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PREFACE TO FIRST EDITION.

It may seem to some persons an act of presumption for a maker of microscopes and microscopic accessories to enter the field of authorship and attempt to supplement the valuable labors which in recent years have made the use of the microscope an indispensible aid in the advancement of science.

To such, if any, I submit, that being a producer of microscopes and their accessories, I have had opportunity to become acquainted with the lack of general knowledge of the fundamental principles of the instrument and the best method of *technique*, even among owners of microscopes. Indeed, with so many complications, with almost unlimited powers and uses of the instrument, the beginner cannot fail to feel the need of a guide and adviser.

In order to accomplish the greatest good, I have started out in this little *Manual* with the supposition that the purchaser, or owner, is a beginner, and absolutely ignorant of the microscope and everything which pertains to it, and therefore have attempted to convey, step by step, in as simple language as I could command, information which will, I trust, lead to ease of manipulation and give both pleasure and profit to those for whom it was specially written.

With these, its purposes and hopes, I beg for my self-imposed labor a friendly reception.

EDWARD BAUSCH.

PREFACE TO SECOND EDITION.

The demand for this book having considerably exceeded the expectations of its author, and the comments on its utility having been so favorable leads to the view that it fills a gap in microscopical literature.

In preparing for a new edition an opportunity has been given for enlarging on some of the subjects and rewriting others, so as to make them conform to the changes which the last five years have brought about in the construction of apparatus.

While it may be true that many of the subjects might be treated much more extensively, the writer has purposely refrained from doing so, because he has considered it beyond the province of his intention and because books giving more extensive information are available.

An intending purchaser of a microscope finds it more or less difficult to make a suitable selection, and while it is always best to consult an experienced microscopist, the writer has endeavored to convey information which, he hopes, will aid in this direction.

May, 1891. THE AUTHOR.

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SIMPLE MICROSCOPES.

Purpose of the Microscope.—The Microscope is an instrument which magnifies objects, so that we are better able to examine their structure than is possible with unassisted vision.

Kinds of Microscopes.—Microscopes may be divided into two classes—simple and compound—the difference between the two being that with the former the object is viewed directly, while with the latter a magnified image is observed ; while the first shows the objects in their true position, the latter shows them reversed, so that what is right in the object is left in the image, and when an object appears to be moving in a certain direction, the movement is in reality the reverse, and must be moved accordingly to keep it in view.

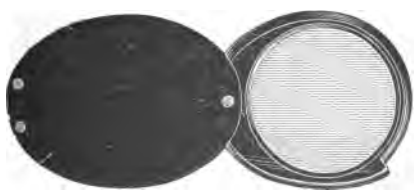


Fig. 1.

Magnifiers.—Simple microscopes are usually termed magnifiers, and, when consisting of one or more lenses, always remain simple. The most com-

mon are those with one or several double-convex lenses (Fig. 1). The shorter the radii (the more curved the surfaces) are in these, the greater will be the magnifying power, and the higher this is, the less of the object's surface can be seen at once. Each additional lens increases the magnifying power in proportion to its curvature. The distance between the lens and the object, when this is seen most distinctly, is called the *focus;* at the point where the object is most distinct, the lens is said to be *in focus;* when indistinct or blurred, *out of focus.*

Magnifying Power.—Unless a microscope is known to come from the hands of a reliable firm, any claim as to magnifying power should be accepted with reserve. In former years, when the country was overrun with cheap foreign productions, the most fanciful claims were made in this direction. It is evident that a lens magnifies an object equally in all directions; this is said to be in *areas*, and is the square of the *linear*, so that if an object is magnified 4 times in the linear, it is 16 times in area. The commonly accepted term to express magnifying power of simple, as well as compound microscopes, is in *diameters* (*linear*). A single lens of 1 inch focus magnifies about ten diameters; one of 2 inch focus, about 5 diameters; one of ½ inch focus, 20 diameters, and so on. In a lens of high magnifying power, the focus is ordinarily about twice the diameter, so that if a lens is ½ inch diameter its focus is about 1 inch. This may, however, be more accurately determined by pro-

jecting, say a flame or window frame, upon a white piece of paper; the distance between the paper to the center of the lens, when the image is most distinct, is its *focal distance.* When a lens is two inches or more in diameter, it is usually termed a *reading glass.*

Using Magnifiers.—In using magnifiers the lens should be held close to the eye and such a position taken that the object will receive the best illumination. In the lenses of equally convex surfaces, it is immaterial which side is held toward the eye; but when plano-convex lenses are used, the plane side should always be toward the eye, as it gives the flattest *field.*

Aberrations.—Two factors arise which prevent the advantageous use of more than about 25 diameters in magnifiers; they are called the chromatic and spherical aberrations. The first is the term employed when the object is apparently fringed with color, predominently blue and yellow; the second, when all but the central portion of the lens shows the object indistinctly; these faults increase with the magnifying power. In the case of a combination of several lenses, they may partially be overcome by interposing an opaque plate with a small opening, called a *diaphragm*, between them, which cuts off the outer or marginal

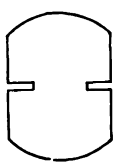


Fig. 2.


Fig. 3.

rays, or the lenses may be made of a smaller diameter. An incision may also be cut into the glass equally between the two surfaces, when from the name of the inventor, it is called a *Coddington*. Fig. 2 shows a section of a Coddington, while Fig. 3 shows it in its mounting.

Achromatism. — The most approved method, however, for eliminating these appearances, is by the use of one or two concave *flint glass* lenses in connection with the double convex *crown glass* lens. When the color or chromatic aberration is thus removed, the lenses are said to be *achromatic*, and when both the chromatic and spherical aberrations are avoided, the lens is called *aplanatic*, and is then said to be *corrected*. An achromatic lens, composed of one flint and one crown glass lens, is called a *doublet* (Fig. 4) ; one with two flint glass lenses and one crown glass is called a *triplet* (Fig. 5). The latter is the best form, as it gives the highest correction ; such a lens (it is thus called from the the fact that the lenses are cemented together and act like one) may be held with either side toward the object with equally good results, and may also be held at quite an obliquity, without loss of definition ; this feature is important, as it is almost impossible to give a lens a theoretically correct position to both the eye and object with the unaided hand.

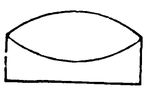


Fig. 4.

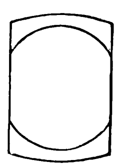


Fig. 5.

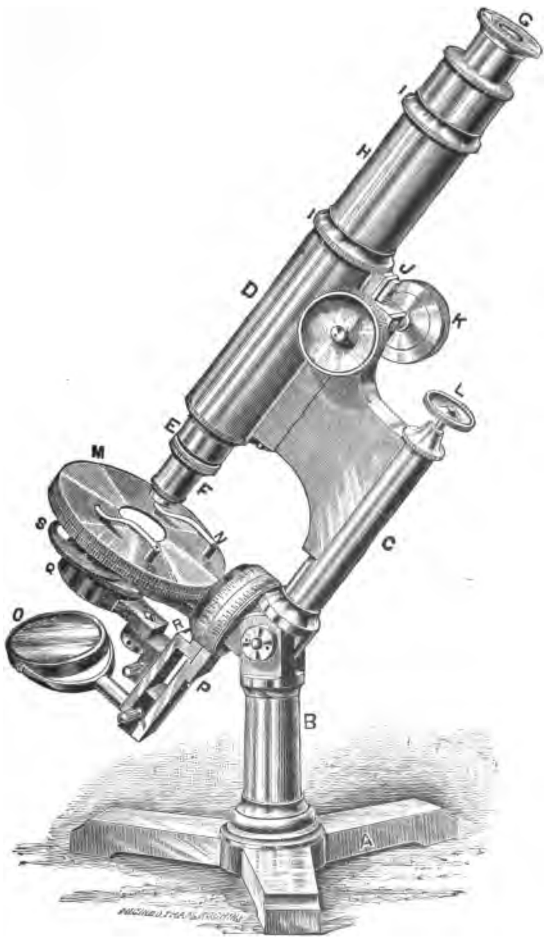


Fig. 6.

THE COMPOUND MICROSCOPE.

As was previously stated a magnified image is observed in the Compound Microscope. Any two lenses, one of short, the other of long focus, placed sufficiently far apart, will attain this object, and this was for years the method of its construction.

On any microscope, whether simple or compound, the difficulty of holding it or the object steady during observation increases with the increase in magnifying power, and in the compound form with only a moderately high power, it is utterly impossible to retain sufficient steadiness to make any reliable observation. Mechanical contrivances thesefore became a necessity, and were applied in the earliest constructions of the microscope. They were all made to fulfill the following three conditions: A platform for holding the object; a means of adjustment for properly focusing on the object, and a mode of suitably illuminating the same.

From what may now be called a crude attainment of these three purposes, the construction gradually became more complex. Many additions have been made which have proven useful and have remained, while others have been discarded, and these have led to the present construction of the microscope.

While certain parts are necessary to make up a modern instrument, no one design of construction is followed. The forms are innumerable, each maker following his own inclination in variety, design, number of parts and material. For the latter brass predominates, although bronze and iron are used to a considerable extent. The first two metals are usually highly finished, and as they easily tarnish in this state, are protected by lacquer, which is not only serviceable in this direction when well done, but offers a means of ornamentation. The latter metal is covered with a heavy coating of japan and being an intense black is on this account often recommended by instructors as being agreeable for the eyes. The entire apparatus is called a microscope, whereas, without the optical parts, it is termed a *stand.*

Description of Parts.—As it is necessary for the student to become conversant with the terms of the various parts and to understand their use, we give an illustration (Fig. 6) with letters, and append a list giving the names.

A. Base on Foot.—This is the foundation of the instrument. It usually rests upon three points (or should do so) and is of such a weight that it keeps the instrument firm when it is in an upright or inclined position. The revolving plate, when this is provided, by means of which the upper portion of the instrument is revolved, without changing the position of the base, is considered a part of it.

B. Pillar.—It is that portion which is fastened to the base and may be one or two, according to the construction of the stand. It carries upon its upper end the *joint* or *axis.*

C. Arm.—This is connected with the pillar by the joint and supports all the worhing parts of the instrument.

D. Body.—This is the tube-portion to which the optical parts are attached.

E. Nose-Piece.—This is an extra piece which is attached to the lower part of the tube.

Society Screw.—This is a standard screw which is cut into the nose-piece, and is called so from the fact that it was first established by the Royal Microscopical Society of London. It is also called the *universal* screw, and is in general use in this country and England ; it has lately been adopted by some firms on the Continent of Europe.

F. Objectives.—This is screwed into the nose-piece and is called so because it is nearest the *object.* It is the most important of the two optical parts (of the microscope proper) and upon its perfection the distinctness of the image and therefore the value of the instrument almost entirely depends.

G. Eye-Piece or Ocular.—It is called so because it is nearest the eye and is the remaining optical part. It magnifies the image given by the objective. This and objective will be treated more fully later on.

H. Draw-tube.—This is that portion of the body which moves in the outer sheath and which receives the eye-piece. It is provided for the purpose of attaining different lengths, variations in magnifying power and as a matter of convenience while working.

I. Collar.—This is a ring which is attached to the draw-tube and is usually provided with a *milled edge.*

J. Coarse Adjustment.—This is a provision for moving the body quickly back and forth for adjusting the focus approximately. It is done by a sliding *rack* and stationary *pinion* (not shown in cut) or a sliding body in an outer sheath.

K. Milled Heads.—These are attached to the shank of the pinion, which is revolved by means of them and are usually large to give sensitiveness to the movement.

L. Fine Adjustment.—This is slow moving and serves to get an exact focus. It is attained by a fine thread, provided with a milled head, and acts upon the body, either directly or by levers. This as well as the coarse adjustment should be extremely sensitive and should not have the least side or lateral motion. The fact that either of them have it, is evidence of poor workmanship.

M. Stage.—This is the portion on which the object is placed for examination and is attached to the arm.

N. Clips.—These are two springs which are attached to the upper surface of the stage and serve to hold down the object.

Centering Screws.—These are provided for moving the stage in different directions to bring the center of its revolving motion in the center of the field.

O. Mirror.—This is used for reflecting and condensing light upon the object. As a rule two mirrors are used, one plane and the other concave. The first gives a comparatively weak light, while the second concentrates it and gives it more intensity.

P. Mirror-bar.—This carries the mirror and by a sliding arrangement allows the variations in distance of the mirror to the stage; it also swings in a circle around the object in order to illuminate it from any direction.

Q. Sub-stage.—This is a ring below the stage to receive various accessories which may be required. It is sometimes fixed to the stage but in the best instruments it is separated from it and is provided with an adjustment to vary its distance from the object.

R. Sub-stage bar.—This receives the sub-stage and permits its adjustmeut. In modern American instruments this, as well as the mirror-bar, is on an axis in the plane of the stage, so that whatever position they may be in, relative to the object, the distance from this to the sub-stage or mirror does not vary, except when made to do so.

S. Diaphragm.—This is a perforated, revolving disk, attached either to the stage or sub-stage. It

has holes of different sizes so that the amount of light from the mirror may be modified.

Optical Axis.—This is an imaginary line which passes from the center of the eye-piece through the body, objective, stage and sub-stage to the mirror. Whatever lies in it is said to be *centered.*

Object.—That which is examined and placed upon a slide.

Slide.—This is a thin plate of glass, generally 3 inches long by 1 inch wide.

Cover Glass.—This is an extremely thin piece of glass, round or square, which is placed upon the object, either for flattening or preserving it, or both.

Classification of Microscopes.—Up to within recent years microscopes have been divided into two classes: the Jackson and Ross models. While the latter was for many years very popular, particularly with the English makers, it has been almost entirely superseded by the Jackson form, and with good reason. In the former the means of adjusting were provided, as near as consistent with the construction, to the body or tubes, whereas in the Ross they are placed at the back or more distant point in the instrument, thus increasing by means of the connecting arm the faults which might exist in the adjustment.

A certain form of instrument which at the present is very popular and called the Continental pattern, from the fact that it was made originally by the

manufacturers on the continent of Europe, is a combination of both the Jackson and Ross models. Whereas, the coarse adjustment when consisting of a rack and pinion is placed closely to the tubes, the fine adjustment is placed on the arm and although being dissimilar from the original Ross in being higher, it nevertheless has the disadvantage of magnifying any lost motion in the adjustment by means of the connecting arm. Considering the fact that the Ross form alone is almost obsolete and many instruments of the present day are a combination of both forms, it appears to the writer that their designations have lost their value.

There is another direction, however, in which microscopes are divided into two classes, which is of far more importance, and affects their utility in a much higher degree. The writer does not know that instruments have been so classified by others, and knows that the subject has been given no important significance.

In the Continental form just mentioned, a short tube from 160.0 to 170.0 mm. (6.3 to 6.7 inch) is used, whereas in the English form, and this is largely followed in America, the length is from 8½ to 10 in. (216 0 to 250.0 mm.). The short tube of the European makers offers no optical advantages, but is mainly used to contract the height of the instrument to as great an extent as possible, as this is the vital point throughout its construction.

At the last meeting of the American Society of Microscopists a committee was appointed to consider the tube lengths as well as other subjects to be mentioned hereafter and reported in favor of the adoption of two standards for tube lengths, 160.0 mm., or 6.3 inch for the short one, and 8½ inch, or 216.0 mm., for the long one. The American makers have adopted these two lengths and we believe are generally following them. Practically, there are no advantages in one or the other, except, perhaps, in so far as the short tube might be considered advantageous, but optically this recommendation of the committee is far reaching, because an objective, particularly in giving considerable magnification, when constructed to be used with a certain tube-length, should be used with it only. When used with the other standard it will fail to give satisfactory results. This subject, with the optical results, will be touched upon again later on.

Mechanical Parts—As there will be but little occasion to recur to the mechanical portions of the microscope, we take the occasion to speak of them in a more extended way in this connection.

Stage.—This may be divided into three classes, ordinary, semi-mechanical, and mechanical.

The first consists of a plain plate, either round or square, on which the slide is held by means of the clips. As the microscope is used principally in an inclined position, the pressure exerted by the clips

must be sufficient to retain the slide firmly in position. This causes a certain amount of friction between the lower surface of the slide and the upper one of the stage, which at times is very annoying, in that it is difficult to move the object to a point desired, with certainty. The motion is "jerky" and disagreeably harsh unless the stage is very clean.

A large number of plans have been devised to overcome this difficulty, which have proven very efficient. They may be termed semi-mechanical, in that the fingers do not come in direct contact with the slide. Usually glass is brought in contact with the metal, but so arranged as to offer as few and small points of contact as possible.

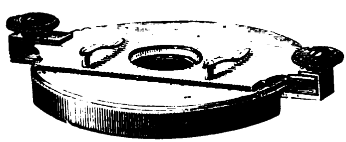


Fig. 7.

In Fig. 7 we show a so-called glass-stage and slide-carrier, in which the stage is merely for the support of the slide-carrier. This latter is arranged to receive the slide and rests upon the glass by small points. At its ends are two projecting tongues which are bent downward and inward and act as springs against the lower surface of the glass plate. While the movements must be carried out by the hands, they are smooth and steady and work with ease, even under high powers.

The mechanical stage is in every sense mechanical. The hands do not come in contact with the slide except to place it on the stage. Two movements are usually provided at right angles to one another, either both with rack and pinion adjustment, or one with rack and pinion while the other has a screw motion. Examinations can be carried out with ease and reliability under the highest powers. The main value of the mechanical stage is that systematic examinations over the entire surface of an object can be carried on.

Mirror and Mirror-bar.—The proper illumination of an object is an important feature, and although there are numerous accessories for properly accomplishing this, which will be spoken of later on, the mirrors alone are effective agents when properly constructed and applied, particularly when no high magnification is used. The plane mirror is usually used on very low powers, and reflects light in about the same intensity as its source. The concave mirror, however, is intended to concentrate the light so that all the rays which strike its surface are reflected toward its optical axis and come together at some point above it, and the rays from the surface being contained within a comparatively small space, cause an increased intensity, This point is called the *focal point*, and is usually arranged to coincide with the opening of the stage when parallel rays, such as from the sky, are used. When the source of light comes considerably nearer to the mirror, as for

instance from a lamp and the rays are diverging, the *focal distance* becomes considerably longer, and when very close may be twice as long. Some of the intensity is lost in consequence, as well as the degree of convergence. For this reason mirror-bars are so arranged that the distance of the mirror from the stage may be varied to accommodate the variation in the source of light. While this is of considerable aid, there is not sufficient room for a complete accommodation, with the result that, under certain conditions, the utmost effectiveness of the microscope is not obtained.

Diaphragm.—This is provided for regulating the amount of light. While the mirror should work to its utmost capacity, it very often occurs that for certain investigations a profuseness of light is more harmful than otherwise. When too much light exists, objects are said to be drowned in it, and often makes it impossible to determine structures. An intelligent use of the diaphragm is of great service.

Besides the revolving diaphragm there are a number of other forms which may be said to be better—for instance, the so-called cup diaphragms, which require a separate piece for each aperture and which are held by a special sub-stage receiver. Then the dome diaphragm, which is a new application of the ordinary revolving diaphragm. It consists of a sub-stage fitting having a dome to which is fitted a curved revolving diaphragm

The ideal regulator of light is the Iris diaphragm consisting of a series of overlapping blades placed

around a central opening, the size of which may be varied by means of a lever or milled edge. In the ordinary revolving form the aperture is of necessity at some distance from the object, and does not fully control the light on account of the stray rays, which the other three forms accomplish.

OBJECTIVES AND EYE-PIECES.

Although considerable magnifying power may be attained by the use of two single lenses arranged in a compound form, there is no advantage in it, from the fact that the faults in the lenses are correspondingly magnified, and these are so considerable that they destroy what it is the purpose of the microscope to give—a distinct image.

Objectives, Classes.—Objectives may be divided into two classes, *dry* and *immersion;* in the former no intervening medium except air exists between the cover and objective, while in the latter a fluid is used to connect the upper surface of the cover to the front surface of the objective. The use of immersion fluid has several advantages, the first of which is that the objective may be made to give better performance, as will be explained later on; the second is that more light will be transmitted, as there is less loss of it by refraction.

It should be understood however that no advantage will be gained by using immersion fluid with a dry objective. It does not increase its effectiveness one particle, on the contrary it detracts from its quality. When it is stated that an immersion objective has a

greater capacity, it is with the understanding that it is so constructed as to give this result.

While many immersion objectives are constructed to work both as dry and immersion, such a plan cannot be said to be advantageous. Such objectives may be made to work well in one direction and be of indifferent quality in the other, or may be of medium grade both ways. There is no question that the best plan is to have each objective selected with a view to a specific purpose and use it for this purpose only.

There are two fluids in general use at the present time, *water* and *homogeneous fluid*. The latter expression means *of the same kind*, and refers to the fact that the fluid has about the same refractive and dispersive power as glass, so that when this fluid fills up the space between the two surfaces of glass, a ray of light passes through the three mediums as if they were one body.

The two large classes of dry and immersion objectives may again be subdivided into two classes—objectives for long and short standard tube. As followed by some firms at present and what it is hoped will become a universal custom in time, each objective is marked for the tube-length for which it is corrected and with which it is assumed it will accomplish the best results.

Objectives are sometimes called *powers*, and in this sense are divided into three classes: *low*, *medium* and *high*. Dr. Carpenter classifies them as follows: *low powers*, 3 inch, 2 inch $1\frac{1}{2}$ inch, 1 inch, $\frac{2}{3}$ or $\frac{3}{4}$ inch;

medium powers, $\frac{4}{10}$ inch, $\frac{1}{2}$ inch, $\frac{1}{4}$ inch, $\frac{1}{5}$ inch; *high powers*, $\frac{1}{6}$ inch, $\frac{1}{8}$ inch, $\frac{1}{10}$ inch, $\frac{1}{12}$ inch, $\frac{1}{16}$ inch, $\frac{1}{20}$ inch, $\frac{1}{25}$ inch.

As the objective is the most important of the two optical parts, it follows that this must be as free from faults as possible and all that human ingenuity and skill can devise is utilized to attain this end. The advance in the perfection of the objective has been step by step and each era was at the time considered by many authorities the limit to further improvement. Each advance was signalized by a marked opposition and disbelief of its possibility. It is therefore of inestimable credit to the pioneer objective-makers, and notably among these two Americans, who by quiet but stubborn application disproved previous claims and opened the way to further improvements. A theoretical limit has been fixed on the capacity of the microscope, which according to our present knowledge can not even be reached.

While the introduction of water immersion made it possible to obtain higher optical results than with the dry objectives, as will be explained later on, the homogeneous immersion offers still greater possibilities in this direction, and the advantages are so pronounced that the former are gradually coming into disuse, although for certain kinds of work they will be preferred and used by many persons. At present homogeneous fluid is made of either thickened glycerine or cedar oil, and great care is required in keeping

the front of objectives and cover glasses properly cleaned, in which respect water has the decided advantage.

It might be stated that such high power as $\frac{1}{25}$th and $\frac{1}{20}$th are very rarely constructed at the present day, and the $\frac{1}{16}$th may be considered the maximum, while the $\frac{1}{12}$th is that most ordinarily used. This power will give all the optical advantages, while higher powers involve so many mechanical difficulties as to increase the cost of production very considerably, and as a rule detract from the optical qualities.

A modern objective of the highest capacity may be considered a work of art, and there are a few productions of the human hand which exact so much untiring application, ingenuity and skill.

Systems.—An objective is said to consist of systems which may vary in number from one to four and five ; two and three are however mainly in use. They are the individual portions consisting of one, two or three lenses, which when more than one, are cemented together and make up the objective. An achromatic single system may consist of two or three lenses, and a three or four system objective may consist of as many as seven or eight lenses. The systems are called in their order: *anterior* or *front*, *middle* and *posterior*. When one consists of two lenses it is called a *doublet*, when of three lenses a *triplet*. Thus in Fig. 8, A is

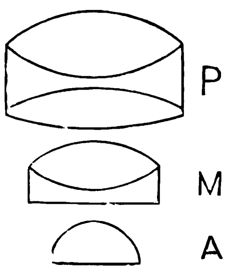


Fig 8.

the anterior, M the middle and P the posterior systems; thus also A is a single system, M a double and P a triple one.

The various features which must be considered as determining the quality of an objective are: angular aperture, achromatism, resolving power, flatness of field, penetration, working distance and magnifying power. Although these attributes may be considered separately, some of them go hand in hand. The presence or extent of one necessarily involves or precludes another.

Angular Aperture.—The angle which the most extreme rays, which are transmitted through the objective, make at the point of focus, is called its angular aperture, or in short its *angle*, and of all the qualities in an ideal objective, this is the most important. Thus in Fig. 9, D is considered the point of focus, and C D E the angular aperture. The above definition has its limitations, however. While in objectives of proper construction it holds true, there are many in which it is not the case. For instance, an objective may be so constructed that it may transmit a considerable number of rays in

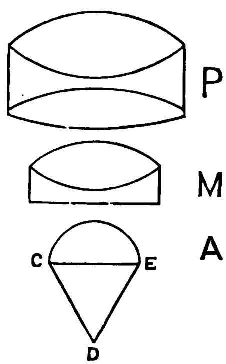


Fig. 9.

excess of those which combine to form an image, and it is evident that these should not be considered as belonging to them.

As there are many objectives of the same power, but of different angular aperture, there are again others of varying power, but of the same angle. Other things being equal, it is the angular aperture of an objective which determines the quality. It is expressed in *degrees*, and is also spoken of as being wide, medium or narrow, although this is indefinite and depends considerably upon the power of the objective; while the angle may be excessively wide for a low power, it may be narrow for a higher one.

For many years the extent to which angular aperture could be carried was a matter of controversy, as was also the use of objectives of wide and narrow angles for different directions of work. It is, however, a matter of congratulation that the question is at rest, although it has served a good purpose in promulgating a better knowledge of the subject.

All objects emit rays, and it is evident that those coming from one point and contained in a large angle are more numerous than those in a small angle; also that as the angle more nearly approaches 180 degrees the rays will be larger in number.

It is assumed that two objects, B and B', are equally bright, and therefore emit the same number

of rays; for the purpose in hand, it is sufficient to consider only those which reach the plane surface of the large lens; if an equal space is imagined over B', the same number of rays will be contained in this;

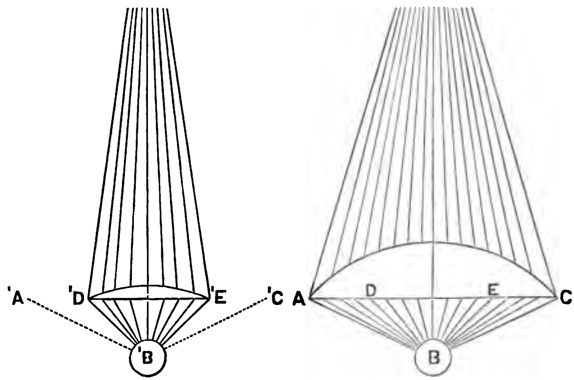


Fig. 10.

therefore, the cone contained in the angle A' B' C' will contain as many rays as that contained in A B C; but as the lens D' E' is considerably smaller than A C, only as many rays can enter it as are contained in the angle D' B' E'. As the rays contained in the angle A B C and D' B' E' are carried through the two lenses, which are supposed to be of the same magnifying power, the image formed by A C will be considerably brighter than that formed by D' E', and will therefore show more of its structure, as will be shown hereafter.

While this is not a theoretically correct explanation of angular aperture, it will serve the purpose of showing its effect. As increase in aperture means increase of resolving power and it will be seen that every degree thus added increases the effectiveness of the objective. It is in this direction that modern advance has been signalized. Whatever views may be held on the advisability of narrow or wide angle objectives, the optical standard of excellence depends particularly on this quality.

Objectives of the same angular aperture, but of different magnifying power (within ordinary limits), will show the object equally well, provided they are otherwise of the same quality, and it is also true that in objectives of the same power but unequal angular aperture, the one of wider angle will show an object more brilliantly than the other, and, if the difference be considerable, will show structure of which no trace can be found with the narrow angle. These are facts which are based upon natural laws, but there are other conditions to be considered in connection with them, which will be treated hereafter.

It very often happens that objectives from different makers, but of the same angle, show a considerable variation ; this does not prove that the above principle is wrong, but is evidence that greater care or skill has been bestowed on one than on the other.

By arranging an objective with an *immersion front*, its angular aperture may be considerably increased over that of a *dry front*, and this explains why better

results may be obtained with the former than with the latter.

Prof. Abbe has introduced a new mode of determining and naming aperture. He calls it Numerical Aperture, and this expression is now generally adopted. Thus, 1.0 of numerical aperture is equal to 180 degrees in air or about 82 degrees in homogeneous immersion, and has a direct relative value to the resolving power of the objective. A complete aperture table may be found in the Proceedings of the Royal Microscopical Journal and larger works on the microscope.

Achromatism.—As has been stated before, when single lenses are made to give a high magnifying power, the chromatic and spherical aberrations prevent corresponding advantages ; and as the objective gives the image which is magnified by the eye-piece, it is evident that if they exist in it, they are increased by the ocular, and that especial care must be given to exclude all faults as much as possible from it. Even with the use of flint glass, it is impossible to free the objective entirely from color ; there will remain a residue of green and purple, and these colors will fringe the object. These are called the *secondary spectrum*, and their presence in an objective is usually evidence of the highest correction.

The amount of color in an objective depends somewhat upon the power of the eye-piece, and becomes more visible as a higher power is used. Color outside of the secondary spectrum is not always preju-

dicial to an objective; for, if in two, one shows the structure of an object with a slight amount of it, the other does not show the structure but gives a nearly colorless image, it goes without argument to say that the first gives the best results and is therefore preferable.

Within recent years new kinds of glass have been found by means of which the amount of remaining color has been reduced, and objectives constructed with it are called apochromatic.

If on increasing the distance between the objective and object the latter shows a marked blue color, and when the distance is decreased a yellow-red color, the objective is chromatically *under-corrected;* if, however, the conditions are reversed, if the object shows a yellow color when the distance between it and the objective is increased, and blue when decreased, it is *over-corrected.*

Resolving Power.—This is the quality in an objective by which we are enabled to see the intricate structure and finer details in an object. It depends upon the amount of angular aperture, the correction of the chromatic and spherical aberrations, and of course upon the perfection of the mechanical work. The power of resolving in an objective is indicative of the perfection of the microscope, for it is almost entirely dependent upon it for its quality.

When an objective is said to *resolve* a structure or a certain number of lines, it means that it shows them under certain conditions of light. It may

resolve *easily* or only *glimpse* them—the latter when they are hardly to be distinguished. The angular aperture of an objective indicates the resolving power, and the theoretical capacity of every degree has been mathematically determined. However, this standard is only reached approximately and to a varying extent. It is not by any means said that every objective of a certain angular aperture will have a corresponding resolving power; it is at this point that the acute accuracy of work and superior judgment of the optician in making proper corrections will invariably give the best results.

Minute structure such as bacteria can only be seen by objectives having high numerical aperture, and these are absolutely necessary in modern investigations. The many recent discoveries can only be attributed to the increased resolving power.

It is an error to suppose that the resolving power may be improved by merely increasing the magnifying power. It is an invariable quality of an objective and has a fixed limit. The extent to which it may be approached depends upon the nicety of manipulation, but no amount of increase in magnifying power by the eye-piece or any other means will carry it beyond it; on the contrary, it will lose in this respect if carried beyond a certain point.

Flatness of Field.—The *field* in a microscope is that portion which is observed in the eye-piece, and its flatness may be observed when focused on a flat object—preferably a micrometer. It is said to be flat

when all portions of the object are seen over the entire field at once without further focusing. When

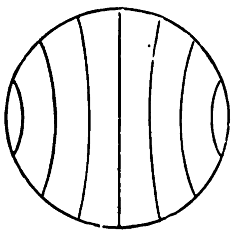


Fig. 11.

not flat, it will be found that as the image approaches the edge of the field it becomes more and more indistinct, and that the objective must be correspondingly adjusted ; in many cases it remains indistinct or blurred, and this may be considered the most serious fault. In the case of looking at straight parallel lines, such as in a micrometer, they will appear to become more curved as they near the edge, as shown in Fig. 11.

Flatness of field mainly depends upon the correction of the spherical aberrations, and as under the best conditions the latter cannot be entirely eliminated, it is impossible to attain absolute flatness, except with eye-pieces especially made for this purpose. It may, however, also be due to a faulty eye-piece ; in this case it can fairly be determined, by observing whether it shows equally in different objectives. With beginners, especially, it is usually most complained of, owing probably to the fact that it is the most easily noticable. It is a desirable quality and indicates to a considerable degree the quality of objectives. While it is impossible to obtain absolute flatness, the optician's effort is to obtain the nearest approach to it. As a quality in itself, without regard to resolving power, it is most easily obtainable, but in connection with this quality it becomes difficult to acquire.

Penetration.—This is the quality which enables us to look into an object—to observe different planes at one time. In the mind of the writer, it is of no special importance, or at any rate not as much as is claimed for it, and if desired is easily attained. It depends upon magnifying power and angular aperture, and decreases with the increase of either of these. Objectives are generally not constructed with any reference to it; it is a natural consequence of certain conditions.

Penetration and resolving power are antagonistic, or at any rate in an inverse ratio, and can only be combined to a certain extent. In two objectives of the same power and aperture, one cannot have penetration as a special feature and the other resolving power; they will be almost similar in these qualities, provided that they are similarly corrected. However, if they are not similar in their angular aperture the one of small aperture will have more penetration than the other. In objectives of the same angle but different power, the one of low power will have in itself more penetration; it will be similar in its action to the eye, which, when an object is close to it, can distinguish but one portion of it distinctly, while, as its distance to the eye is increased, can distinguish various parts of it lying at different distances, and will finally see other objects outside of it. By looking at an object at 5 feet distance, only this can be seen plainly; but, at 10 feet, others quite a distance in front or back of it can be seen as well.

Working Distance.—This term, strictly considered, is an invariable quality of the objective, and is the distance between the front lens in the objective and an uncovered object, when the objective is in focus and is corrected for that object. All objectives require a certain amount of projecting metal to protect the front lens, and this with a certain thickness of the cover-glass lessens it. In objectives with fixed mountings this may be, and with thick cover-glasses is considerable. As it is comparatively unimportant, however, for the working microscopist to know the working distance *per se* of his objectives, but of considerable moment to know what the actual space between the objective and cover glass is, it would be well, in the mind of the writer, to express it as *available* working distance.

In objectives of low and medium power, it is of little consideration; but where it must be expressed in $\frac{1}{100}$ or $\frac{1}{1000}$ inch, it becomes a matter of importance.

Working distance is spoken of as being *long* or *short*, and varies not so much with the power as with the angular aperture; generally the working distance decreases with the increase in angular aperture, and becomes greater as the aperture becomes smaller; it it was for a long time considered that these two properties varied according to a fixed rule, but this at the present time is not considered to be the case. While in objectives of the same aperture it may vary considerably, it may in others of different aperture be so that the higher one may have the greater working

distance. The skill of the optician must in a considerable manner determine the amount of it.

It will be seen from the above that working distance stands in no direct relation to the focal distance of the objective, neither to its nomenclature or rating, and, it may be added, that it is never as great as the focal distance of a single lens of the same magnifying power.

As may be imagined, there are a variety of opinions as to what constitutes long or short working distance in a certain objective. No definite rule can be laid down for this, as it is conditioned by the skill and requirements of the manipulator. Although it is an important factor, the idea that it should in all cases be as great as possible, is erroneous, for, while it may be true in a dry objective, it may be the cause of annoyance in one with immersion. On several occasions it occurred in the experience of the writer that after an objective had been completed, it was found that its working distance was so large that the immersion fluid would run out from between the objective and the cover-glass when the instrument was inclined, and it was necessary to change the objective with a view to decreasing its working distance, in order to allow its convenient use.

Magnifying Power.—This is a question of vital importance in a microscope, not so much as a quality for itself, as in connection with the resolving power. The inquiry should not be simply how many diameters an instrument will magnify, but what the precision

and extent of its definitions are under a certain magnifying power. If a high magnifying power is all that is desired, this may be obtained to an almost unlimited extent by means of simple lenses which may be procured at a small pecuniary outlay; but these do not give a distinct image nor do they make structure visible, which, be it remembered, is the purpose of the microscope to do.

The normal eye can distinguish from 200 to 250 lines to the inch, and in a microscope such magnifying power should be used, which will apparently bring the structure which is sought after *at least* up to this figure. In illustration take a ½ inch objective of 98 degrees and a 1½ inch eye-piece. An objective of this kind properly corrected, resolves *pleurosigma angulatum*, in which the average lines are 60,000 to the inch. With the above eye-piece it is utterly impossible to see them, while if it is replaced by a ¾ inch or ½ inch, they can easily be distinguished. This is not owing to any peculiar quality of the eye-piece, but merely to the fact that by increasing the magnifying power, the dimensions of the object have been increased to such an extent that the lines have apparently been separated and become visible to the eye.

Beginners as a rule are apt to use too much magnifying power or *amplification*, and often attempt to view a large surface with an objective which will show but a small part of it. It must not be forgotten that the apparent field of view is decreased as higher powers are used, and that a low power will give a

better impression of a large, coarse object and its relative parts, not only because it makes a larger surface visible, but because it has more penetration.

In objectives of the same power, but of different angular aperture, the magnifying power and field will always be the same.

The following table which has been compiled will probably be of assistance to the beginner. After he has become better acquainted with his instrument his judgment will dictate to him what to do.

A power of 25 diameters will show a surface of about $\frac{1}{5}$ inch diameter.
A power of 50 diameters will show a surface of about $\frac{1}{10}$ inch diameter.
A power of 100 diameters will show a surface of about $\frac{1}{20}$ inch diameter.
A power of 500 diameters will show a surface of about $\frac{1}{100}$ inch diameter.
A power of 1000 diameters will show a surface of about $\frac{1}{200}$ inch diameter.

This table is approximately correct with a Huyghenian eye-piece; with a Periscopic almost double the amount of such surface will be shown.

Magnifying power may be obtained by the eye-piece or objective and the desirability of using one or the other for this purpose was for many years a matter of spirited discussion, but it is now generally conceded that increased power should be obtained by increasing the power of objectives and not go beyond the equivalent power of 1 inch in the eye-pieces.

Objectives of the same angular aperture, but of different power, will give identical results by bringing them up to the same magnifying power, unless the

difference is considerable. In both objectives and eye-pieces the lenses decrease in size with the increase in power and consequently give less light; and while this one objection exists in the objective an additional one occurs in the eye-piece, in that the eye must be brought closer to the eye-lens and must be kept more strictly in the optical axis, which at a long sitting becomes fatiguing.

Between the 1 inch and 2 inch the choice should be determined by requirements and individual preference. All responsible manufacturers and dealers make up such outfits of stands, objectives and eye-pieces, which experience has taught them are most generally useful.

It is a safe rule to follow in all work on recognized forms (objects of which the structure is known) not to use a higher power than is necessary to properly study them.

Eye-Piece—Huyghenian.—This is now in general use, and consists of two plano-convex lenses. It receives its name from the inventor, who first applied it to the telescope. The *eye-lens* is the small lens nearest the eye, and the *field-lens*, or *collective* as it is also called, is the large one nearest the objective. A *diaphragm* is placed between them, and gives a sharply defined *field*. This eye-piece is also called *negative*, as its focal point is between

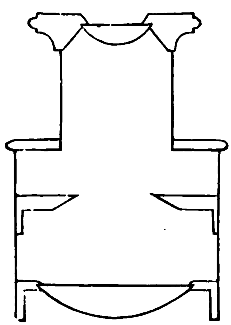


Fig. 12.

the two lenses (at the diaphragm) in contradistinction to a *positive*, in which the focal point is outside of and below the field-lens.

The Continental eye-piece is also a Huyghenian, although it is mounted in a straight tube, in place of the mounting, with the neck as shown in cut. The American Society of Microscopists has recently recommended that eye-pieces be made par-focal, that is, that the equivalent foci coincide and that the par-focal plane correspond with the upper end of the tube. This is an excellent plan, as the focus on the object is maintained whatever may be the change in eye pieces.

Solid Eye-Piece.—This was the invention of the late R. B. Tolles, and also belongs to the class of negative eye-pieces. It is called *solid* from the fact that instead of being composed of two lenses, it consists of one piece of glass, which is cut to a cylindrical form, and on the ends of which the proper curvatures are ground ; the diaphragm is made by cutting a circular groove into the glass at the proper distance between the two surfaces, which is then filled up with an opaque pigment.

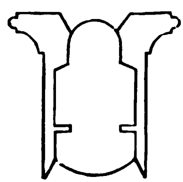


Fig. 13.

These eye-pieces are only made in high powers, as optical glass is usually not of sufficient homogenuity to make low powers, and their cost would be too considerable, without a corresponding advantage. For high powers they are superior to the Huyghenian, in

that they give a better illuminated field, as there is less loss of light by absorption through the glass than by refraction at the two additional surfaces of the eye-lens and field-lens in the Huyghenian.

Periscopic Eye-Piece.—This consists of a triple eye-lens and single field-lens. Its predominant feature is a very large and flat field, with almost all objectives. In this respect it has a considerable advantage over the Huyghenian and Solid. It is positive and therefore well adaped for micrometer work, as it is focused like a magnifier, and its magnifying power remains constant, while with the Huyghenian it is variable, from the fact that the eye-lens alone is focused, thus varying its distance from the field-lens, and consequently the magnifying power.

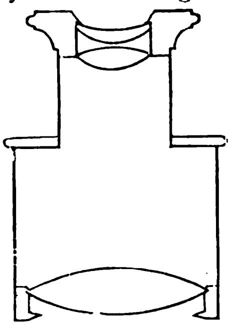


Fig. 14.

Nomenclature.—The rating of eye-pieces was formerly, and is to a considerable extent to-day, by letters. This method, however, is arbitrary, as the letters of different makers have a totally different significance, so that nothing like a standard exists. This fact induced the American Society of Microscopists to endeavor to establish a universal method, and after the matter had been given careful attention for several successive years, it finally adoped the method which rates them according to their magnifying powers, the same as that which has been used in objectives. This gives an approximate idea of the

magnifying powers; thus, an eye-piece marked 1 inch or by a letter signifying the same, shows that it magnifies about 10 diameters; one of ½ inch, 20 diameters, and so on.

Flatness of Field.—Although this depends mainly upon the objective, the absence of it may be owing to a faulty construction of the eye-piece. If it is so prominent as to be easily noticeable, and to the same degree with a number of objectives, it may be ascribed to the eye-piece. It must, however, be remembered that an absolutely flat field has not yet been obtained; it may be closely approached by decreasing the diameter of field to less than its normal size.

Size of Field.—Quite a general but errorneous idea prevails that the size of the tube has an influence on the size of the field. Except in eye-pieces of very low power, or with tubes with smaller than usual dimensions, this is not so. It must be remembered that a Huyghenian eye-piece admits of a definite size of field, and this is regulated by the opening in the diaphragm; the same size of opening is used in all of the same power, whether it is an eye-piece for a large or small diameter.

A misconception also exists as to the definition of field. Such inquiries are often made as: "As we understand it, a wide-angle objective gives a larger field?" but it does nothing of the kind. The angular aperture has no bearing whatever on the size of the field. The *field of view*, or that which is shown of the object's surface, is determined by the power of the objective and eye-piece.

REQUISITES FOR WORK.

It is the intention to make such recommendations in this chapter which, if not absolutely necessary, will be found convenient and will aid in facilitating work.

One of the first requisites for the proper use of the microscope, is a thorough knowledge of its parts and an acquaintance with the optical principles involved. For this purpose the writer earnestly requests a perusal of the preceding pages, and is convinced that in cases where no previous knowledge of the instrument has existed, work will be done with far more ease, in much less time, and with a greater degree of satisfaction. Ignorance of the instrument's capacity may lead to an idea that it is inferior and thus be the means of its final abandonment; and in place of the anticipated pleasure there may arise a feeling of bitterness and disappointment for all future with everything connected with it. There are innumerable cases of this kind and they have induced a belief that it is difficult to acquire a practical manipulation of the microscope, whereas such is not the case when a limited time, properly applied, is devoted to it.

Working Table.—A firm table should be used, preferably one with three legs, as this will always be firm no matter how uneven the floor is, and if it can

be arranged, should be devoted to this purpose only. One with a round or square top of three feet provides ample room. Although not necessary, a table with a revolving top, provided with clamp, is very convenient, as with this two or more persons may make observations without changing their seats.

A very neat arrangement for a table top is that suggested and used by Dr. J. E. Reeves. He places upon an ordinary table three or four thicknesses of white paper and upon these a plate of polished glass as large as the top; this can be procured of almost any glazier at a low price. It is pleasant to work upon and will not soil.

As in almost all cities there is more or less continual vibration from wagons upon the paved streets, the writer suggests an effectual remedy. Take a thin board, say half an inch thick, of a sufficient size to receive the microscope; fasten on the upper side at two opposite ends, cleats of 1 inch square and counter-sink into these through the board four spiral springs of such tension that when they bear the weight of the instrument, the bottom of the board will be about ¼ inch from the table.

Have the working table provided with drawers and arrange receptacles for the accessories, secure from dust, but at a convenient point to reach. When the instrument is not in use put it into its case or cover it in a manner so that it shall be free from dust. For this purpose a large bell glass is best.

Room.—If possible a room should be selected facing the north, as the light in this direction is most constant. It will prove a great saving of time if all or a portion of it can be permanently arranged to receive the entire working outfit. It should also be chosen with a view to its being free from disturbance.

Light.—As stated, the light from the northern sky is most desirable, and that from a white cloud is preferable to that from a blue sky. On account of its intensity, direct sunlight should seldom be used ; but if modified by a white curtain or reflected from a white wall it is excellent.

For lamp light an ordinary flat wick kerosene or student lamp is well adapted. The Hitchcock lamp, from its better combustion is still better, as its color more nearly approaches white. The ideal artificial light is that from an electric light. Gas light is not desirable as it is seldom sufficiently steady.

Position of Light.—The relation of the microscope to the source of light is principally a matter of personal convenience. With daylight it makes little difference whether it is at the front or side of the instrument, although the writer prefers it at the front, as the manipulation of the object does not obstruct it ; but the lamp should be placed at the right or left side within easy reach of the hand for the purpose of controlling it. The writer suggests that the beginner make it a habit at the outset to place it on the side of the instrument opposite to the unoccupied eye, as the tube then places the latter in the shadow.

Which Eye to Use.—In a binocular instrument both eyes are used, but in a monocular only one is used, and it depends upon a trial which is best suited. A large proportion of persons are afflicted with astigmatism, often without knowing it, and when this exists it may be in one eye or when in both, may be to a greater extent in one than in the other. Its presence may prevent the eye from observing fine detail; but whichever eye is found to be best suited should be used. When both eyes are alike it is sometimes advisable to change from one to the other.

It should be made a habit at the outset and strictly adhered to, to *keep both eyes open.* A little difficulty may be found to do this, as the eye which is free will probably observe the objects upon the table; but as soon as the mind becomes fixed upon what it sees in the microscope, this impression disappears. After a time it will be found to require no exertion and will certainly add to the ease and comfort of the manipulator while working.

The Ward Eye Shade, Fig. 15, will prove of assistance in acquiring the above mentioned habit, and ebsides this, effectually excludes the light from the eyes.

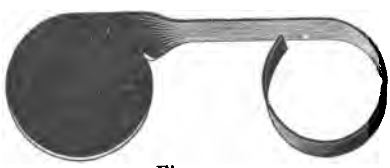


Fig. 15.

It is made of hard rubber and is attached to the tube of the microscope.

Order.—Among the requisites for successfully prosecuting work with the microscope are a strict observance of the instructions, even if they appear superfluous, a systematic way of doing work, and cleanliness. Have a place for every article which is required, so that the hand may immediately be placed upon it; after it has been used clean it before putting it aside; keep strange hands from your apparatus unless you are assured that a knowledge of its manipulation exists.

Material.—Although the purpose of this manual is to be a guide to the intelligent use of the microscope and not the preparation or preservation of objects, it may not be out of place here to enumerate what every owner of an instrument should have at the outset. The first should be a book on objects giving proper instruction on their preservation. There are many of these, and all of them good. Next in order, slides, covers and labels are necessary. As covers are easily broken in cleaning, a larger proportion of them will be necessary.

A cabinet for slides, a large variety of which may be selected from, will aid in starting work in a systematic manner. Forceps and a small pipette are indispensible. For preserving objects, Canada balsam or damar should be purchased, while the other necessary material which may be gleaned from the instruction book is easily obtainable.

When it is intended to do section-cutting a good mechanical microtome, not necessarily expensive, should be obtained at the outset.

HOW TO WORK.

To Set Up the Instrument.—Draw the instrument from the case by grasping the base, and free it from dust. If it has *draw tube*, bring it to its standard length, which is indicated by a ring, by as little of a screw motion as possible; if the draw tube is highly polished, its surface will be best retained by observing the above precaution. See that the *mirror* and largest aperture of the *diaphragm* are in a central position—in the *optical axis.* After being convinced that the *eye pieces* are clean, place one into the *tube;* then remove the *objectives* from their cases and after first having increased the distance between the *stage* and *tube* by means of the *milled heads* of the *pinion,* attach the lowest power to the *nose-piece,* by using both hands, being careful that it is as near as possible in the *optical axis* while screwing it on. Then incline the *body* by placing the left hand upon the *base* and drawing with the right hand upon the *arm;* be careful not to pull on the tube, as it may prove too heavy a strain upon this or the fittings. Incline the body of the microscope until the eye piece about reaches the level of the eye, so that when an observation is made the position is as comfortable as possible; the

neck should not be strained, neither should the chest be compressed. Next place the *slide* with a transparent *object* upon the *stage*, by sliding it under the spring *clips* and get it as near as possible in the center of the opening; for an object anything near at hand, such as a piece of printed paper or cotton fibres will do. Watching the slide, adjust the mirror until it is seen that the light strikes the object; incline the head to the level of the stage, and observing the objective, rack it down to within $\frac{1}{4}$ inch of the object. Again placing the eye at the eye-piece, reverse the motion of the milled heads and observing the *field* continue the upward motion of the body until the image of the object appears in view.

Centering Stage.—If the microscope has a revolving stage, turn this to see whether the object or portion of it lying in the center of the field, remains in the optical axis. It was true when the instrument was shipped, but may have changed during transportation. If not centered, loosen the screw holding it to the arm by means of the steel pin, just sufficiently that by the exertion of a little pressure it can be moved. After having observed first which portion of the object remains stationary during its revolution (this evidently is its center) move the stage so that this point will be in the center of the field, and then tighten the screw. If the point lies outside of the limits of the field, its direction can be noted and the stage moved accordingly. Where *centering screws* are provided in the instrument, this is a simple matter.

If the *coarse adjustment* does not prove sensitive enough to *focus* easily, adjust by the fine adjustment by taking the head of the *micrometer screw* between the thumb and first finger and move toward the right or left as may be necessary.

It may here be said in passing that the rack and pinion should be so well fitted that they should permit the adjustment of low power objectives with the greatest ease, and should work *without the slightest lost motion* with a $\frac{1}{5}$ or $\frac{1}{8}$ inch objective. This point is the criterion of workmanship in an instrument, and if it is found to have the least *back-lash*, or is not perfectly smooth, it may safely be assumed that the instrument is of inferior workmanship.

If the fine adjustment does not act, the screw has either come to its stop, or has "run out," and must be brought into action again ; the range of movement in almost all fine adjustments is quite short and constant care must be taken to keep it at about a medium point. If the object is found not to give a full view or is not in the center of the field, it must be moved on the stage, but it must be remembered that a movement in one direction causes an apparent opposite movement in the field. At first this movement will be in jerks, but after a little practice the necessary sensitiveness of touch is acquired to give it more steadiness.

Illumination. — It should now be observed whether the field is equally illuminated. Too much stress cannot be laid on this point,

as it is one which is easily overlooked and is often the cause of considerable mischief. If the light comes through a window a well defined image of the sash is reflected by the mirror, and with a lower power objective this can easily be seen; unless the mirror is correctly adjusted the field will appear to be crossed by dark bands. In the case of lamp light the flame is reflected and has a similar effect. With high powers this fault is not so easily noticed, and for this reason require the more care; proper resolution may in this manner be partially or totally destroyed. The remedy is either by shifting the mirror or by varying its distance from the object.

More information on this subject is given under the head of Sub-stage Illumination.

Attaching High-Power Objectives.—As the difficulty of properly getting an object or a certain portion of it in the field, increases with the magnifying power, it is a good rule to use the lower power objective as a "finder;" after getting the point to be further examined in the center of the field, remove the objective and attach the higher power, and after following the procedure of focusing as with the low power, except that the objective should be brought almost in contact with the cover, the point will be seen in the field or will be found to be close to it.

This plan of focusing as suggested above is always a good one to follow and is observed by many of the most expert manipulators. In many cases, however, the focus is obtained without this precaution, by

watching the field as the objective is brought down toward the object, but is often followed by disastrous results. Almost any person who has used the microscope for any length of time is without question aware that valuable preparations have been destroyed in this way.

How to Work.—It is now supposed that the instrument is ready for work. To start, it is well for the beginner to provide a few prepared specimens, as these will help him considerably if it is his intention, as it should be, to prepare them later himself. Whatever branch of study he is going to follow, a slide of *pleurosigma angulatum*, dry, will be valuable to practice upon and to determine the quality of his higher power objective. In this latter respect, however, the writer would advise the beginner to guard against expressing an opinion too soon. He knows of many cases where the optician's claims were flatly denied, when often a few words of advice by lettter or a few minutes of intelligent manipulation would resolve the diatom, and would thereafter do it so easily that it became a wonder how it could possibly be avoided.

For a low power objective, the *proboscis* of a *blow-fly* is probably the most suitable and at the same time most interesting object. Place this upon the stage, and, after getting it as close as possible to the center of the opening in it, focus by means of the coarse adjustment. If only a portion of it can be seen and if it is desired to see a larger surface, the length of tube may be contracted by means of the draw-tube.

In this case the object will be placed *out of focus*, and another adjustment becomes necessary. If a higher power is desired, the draw-tube may be extended. Observe whether the field is well illuminated, and if not, bear in mind what has been said about properly adjusting the mirror. If the object appears milky or the light is so intense as to be painful to the eye, which is of usual occurrence to the beginner, the diaphragm should be turned from one aperture to another until a marked difference is seen ; or, the plane mirror should be used. In this connection it is well to state that the above precautions should always be observed with low powers, unless the object is thick. Now use the micrometer screw and note carefully the beautiful structure which is opened to view. After sufficient time has been spent upon this, the objective may be replaced by a higher power and the object by a slide of *P. angulatum ;* focus upon this, being mindful of the suggestions previously given, and do not fail to observe what has been said in regard to a well illuminated field. If lamp light from a flat wick is used, turn the edge of the flame toward the mirror, and use the concave side of the latter. If the diaphragm is in an adjustable sub-stage, bring it as close to the stage as possible, or, whether here or attached to the stage, it may as well be removed for the present. Observe now whether outside of the central rib any lines can be seen upon the surface of the diatoms ; if not, vary the distance of the mirror from the object ; or, if lamp light is used, bring the lamp

closer to or remove it from the instrument in one line, so that the illumination will not disappear. If this does not bring out the lines, swing the mirror-bar from the *central* position into an *oblique* one, on the side opposite to that of the light and readjust the mirror; in doing this grasp the ends of the *mirror-fork* between the thumb and middle finger and move the mirror by the first finger. If the field can not be evenly illuminated, it is evidence that the mirror is beyond the limit of angular aperture in the objective, and it must therefore be brought back until it is. It must here also be noticed that if the diaphragm is still attached to the instrument and does not swing with the mirror, it may also be the means of cutting off light. By means of the micrometer screw carry the fine adjustment back and forth beyond the plane of the object and observe closely whether any lines can be distinguished. It is very probable that they will show; but if not, the cause should be determined. It may be that the magnifying power is not sufficiently great, and in this case a higher power eye-piece should be used, or the cover glass may be more or less than the normal thickness, which would cause a spherical over or under-correction in the objective. In this case the lines would appear when the diatom is not in focus. If the objective is a non-adjustable one, the proper correction may be approximately reached by means of the draw-tube. If the lines appear over the plane of the object, it shows over-correction, and the length of tube should then be decreased, or contrary when the lines show below or

beyond the plane of the object. If the above directions have been followed, the lines cannot fail to be seen with a moderately good $\frac{1}{4}$ or $\frac{1}{5}$ inch objective; but if they are not, the trial should be repeated. Again, be careful to have no obstruction between the course of rays from the mirror to the stage; get good illumination on the object; observe well; keep the instrument in such a position that the object is not illuminated from any other direction than from the mirror.

When the diatoms are *resolved* in this manner, the lines will appear to be *diagonal* in some; *longitudinal* or *transverse* in others, according to their position; and, if the resolution is very good, these lines will further resolve themselves in minute beads of a hexagonal form.

It will now be well to bring the mirror more nearly to a central position; do this at intervals of about 10 degrees, and note the appearance at each decrease of obliquity. It will be found that as the mirrror approaches the optical axis the lines will appear to become more faint, and may disappear before central illumination is reached; in this case it will be well to begin again. An endeavor should be made to make each attempt give better results than the preceding one. Repeated trials will not only impress the various phenomena upon the mind, but will cause a notable improvement in manipulative skill, and thus a better performance in the objective.

Until now we have assumed that *transmitted* light has been used. We will now suppose that the object

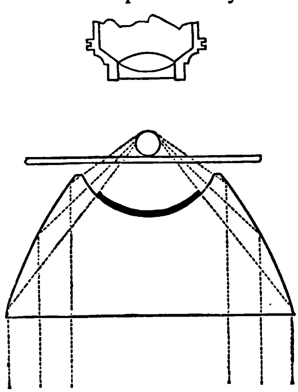
is not sufficiently transparent to use this method ; the object is then said to be *opaque*, and requires a different procedure. We will say that it is desired to examine an insect ; it may be attached to a slide, or, what is better still, may be fastened in a stage forceps (Fig. 16) as it may then be turned and viewed from all sides. The low power objective should again be attached ; after having been focused, it will be found that the light is insufficient to illuminatate it. The mirror-bar should now be swung upon its axis around the stage to a point above it, so it will be at an angle of about 45 degrees to its surface. If a lamp is used and is in the same position as when used with transmitted light, it is probable that the tube of the instrument will obstruct the light, and it is then well to move it toward the front. Using the concave mirror adjust it so that the light will be concentrated upon the object, by watching it directly, and then observe through the tube. If it is not sufficiently illuminated continue to adjust the mirror ; also vary its distance from the object and swing the mirror-bar to a higher or lower point. It often occurrs that, under the best conditions, the need of better illumination is felt ; in this case a *bulls-eye condenser* should be procured. It will be found that this will become a useful and perhaps necessary accessory in work outside of this. Place it close to the instrument and set the bulls-eye between the object and source of light, with the *plane*

Fig. 16.

side toward the object; if an ordinary hand lamp is used, it will be necessary to elevate this to about the height of the eye-piece, and if it is to be used often in this position, a special support should be made for this purpose.

Low power objectives are usually used on opaque objects, but sometimes a higher power is desired. Unless one is constructed with a view to opaque illumination its working distance is usually so short that it will prevent the light from striking the object. A $\frac{1}{4}$ or $\frac{1}{5}$ objective, of 75 degrees, has sufficient working-distance, and its mounting are made conical in the front, so that it will allow it.

Dark Ground Illumination.—This method is not in general use, probably because it requires a special accessory, although it yields beautiful effects. It is accomplished by means of a paraboloid (Fig. 17) which is attached to the sub-stage. As will be noticed in the illustration, the lower surface of the paraboloid is plane, and the light passes through this without undergoing any change. When it reaches the polished parabolic surface it is reflected to one point, according to the simple optical law that the angle of reflection is equal



Ffg. 17.

to the angle of incidence. An opaque stop, which is cemented to the concave surface, prevents the light from passing through the central portion of the paraboloid. The object is thus illuminated on all sides by such an obliquity of light, that it does not pass into the objective; the object stands out in relief, pleasantly illuminated on a dark back-ground. In using the paraboloid, the plane mirror should be used, and it is necessary to vary its distance from the object in order to attain the best results.

Cover-Glass.—Thus far no attention has been given to the use of the cover-glass, although it is an important factor in reaching good results. In preliminary examinations of solid objects with low powers it may be dispensed with; but where fluids are used, whether with low, medium, or high powers, it *should always be used.* A drop or small quantity of fluid placed upon a slide assumes a spherical form, and, on viewing it with a low power, it will be found to give a distorted field, and will cause disagreeable reflections and shadows.

As stated before, medium and high powers have a comparatively short working distance, and the front lenses will be so close to the water, urine, blood, etc., that the capillary attraction will often cause an adherence to the front surface of the objective; besides this, there is such a considerable depth to the fluid that it obstructs the light, requires a great change in adjustment for the various planes, and is usually in such vibration that a sharp focus becomes impossible;

by merely dropping a cover-glass upon it all these objections are overcome.

The above are merely practical considerations, but there are others of a theoretical nature and of as much importance. After a high power objective has been corrected to a certain thickness of cover. any variation, not necessary considerable, has an injurious effect upon the spherical corrections, and consequently upon the resolving power. It is manifest that the quality of the latter will decrease as the variation increases, and when it reaches a point where no cover is used, it may be so considerable as to destroy an accurate perception of what is sought.

In this connection it is considered important to state what thickness of cover-glass it is best to use. As is probably well known, there are three grades, which are designated as No. 1, No. 2 and No. 3. Although they are classified, there is a variation within the limits of different numbers The variation is about as follows: No 1, $\frac{1}{150}$ to $\frac{1}{200}$ inch thick; No. 2, $\frac{1}{100}$ to $\frac{1}{150}$ inch thick; No. 3, $\frac{1}{150}$ to $\frac{1}{100}$ inch thick; According to the prices of cover-glasses, when purchased by weight, the No. 1 give the greatest number and No. 3 the least. It may for this reason be thought that the purchase of No. 2 is most advantageous, but it must be considered that there is a greater proportion of breakage by cleaning, as they are very thin and sensitive. Considered only from a optical standpoint, No. 2 should generally be used, as the medium and high power objections are adjusted

to this thickness and give the best results with the thinnest of these. The same thickness is also used on test objects, but they are generally not of as much uniformity as might be desired. Objectives sometimes have such an extremely short working-distance, that it is necessary to use the thinnest of No. 1, but as these are usually provided with adjustment for correction, their injurious influence is not so much felt. The thickest covers are most comfortable to handle and may be used with low power objectives without much sacrifice of definition.

The writer takes the liberty of inserting in this connection extracts from a paper which he recently read before the American Society Microscopists and which he hopes will give further information on this subject.

"The cover-glass may truly be called a necesssary evil; for, while absolutely required in microscopic investigations, there is no adjunct to the microscope that has been and is productive of so much evil, and has retarded the utilization of benefits made possible by the advance in the construction of objectives so much as it.

"It must be remembered that the majority of objectives will always be dry, and especially so when such improvements, which we hope are still to be made, are accomplished. It is an unfortunate circumstance that with this class of objectives the influence of variation in thickness of cover-glasses is most apparent; but since it is so, we should, if possible, provide an

agency which, eliminating the personal factor of efficiency, will give, under all conditions, results closely equal to those under which the objectives were originally corrected.

"It is surprising to see how little attention is paid to this subject in the large majority of standard works on the microscope. Almost all books give carefully prepared illustrations and descriptions showing the effect on the course of light by the interposition of the cover-glass, and after giving conclusive evidence of its disturbing influence, still, in a general way, say it is of little moment.

"With such statements to guide the microscopist, it is not surprising that the subject should have received so little attention, and that any efforts to lead to improved methods of manipulating objectives should have almost completely failed because of a lack of the true understanding of their need and consequent failure to create interest. The belief is quite general that any time devoted to this subject is wasted and might better be utilized in other directions. I hope to be able to show that this is entirely wrong, and may here say that, while I may be considered an extremist in the other direction, my efforts emanate from the desire to put it in the power of every microscopist to obtain the highest possible results from his optical battery and equal to those obtainable by the optician.

"Outside of the differences of the lengths of tubes used by different makers, which is also of great bear-

ing on the spherical correction of objectives, one is astounded by the difference in standard cover-glasses used by different makers in correcting non-adjustable objectives. With a thickness of 0.10 mm. for the thinnest and 0.25 mm. for the thickest, it is only too apparent that with the additional variation in lengths of tubes it is beyond the power of the microscopist to obtain even approximately the best results from his objectives. More than this, a large quota of the advance made in recent years in the capacity of objectives has been lost.

"The greatest difficulty is met with non-adjustaleb objectives. As is well known, compensation for thickness may be obtained in the proper adjustment of tube length ; but while not all microscopes are suitably provided with draw-tubes, the requisite experience and skill is lacking with a large number of microscopists to properly make the correction in this manner, as well as in objectives specially provided with collar correction. I am sure that microscopists of long experience will bear me out in the statement that results with adjustable objectives depend upon individual skill, and that many such objectives now in use fail to give results corresponding to their capacity. It would seem, therefore, that any system to permit the full utilization of the capacity of objectives should depend on no personal factor—in fact, should be mechanical.

"In an objective corrected for normal thickness of cover-glass there will be spherical over-correction with

thick covers and under-correction with thin covers, the amount of correction varying in a different ratio to the amount of variations from the normal thickness. The chromatic correction will also lose correspondingly, but to not so high a degree. While a devi-

Fig. 18.

ation of a few hundredth millimeters in either direction will, perhaps, not signify, that which occurs in covers classified in price-list under one number is

sufficient to seriously affect and the high powers totally obliterate the definition which under normal conditions it may possess. The microscopist is therefore not obtaining such results as his objectives ought to enable him to obtain, and the efforts of the conscientious optician to provide classified objectives of reliability and similar performance is almost entirely nullified.

"The system which I have devised to aid in overcoming these difficulties depends in the first instance upon a micrometer for measuring the thickness of cover glass. See Fig. 18.

"In objectives provided with cover correction the graduation is so arranged as to read to $\frac{1}{100}$ mm. No matter what the power of objective, the number gives proper correction for a thickness corresponding to it. Thus, with a cover glass of 0.20 mm. the collar of such an objective need merely to be set at 20 to give the proper correction and, consequently, the best results.

"All the other scales give the correct tube length in inches and millimeters for covers corresponding to them, and in this manner offer a ready and definite means of correction. The tube-lengths required for the thinnest and thickest covers are so extreme that probably no convenient means for obtaining them can be practically arranged, but they can be so approximately if not entirely. At any rate, the micrometer will detect the requirements before using the covers,

and those deviating considerably from the normal can be used on objects for use with low powers only, in which case the effect will not be very appreciable.

"In this system I do not overlook the fact that variation in tube length involves a variation in magnifying power; but, except in cases where micrometers are used, I consider this of secondary importance, as it always is in comparison to results obtained in resolving and defining power.

"This system involves four conditions:

First.—That all cover glass be measured before using them, and that the thickness be noted on the preparation.

Second.—That for convenience all draw-tubes be marked in inches or millimeters or both.

Third.—That adjustable objectives be corrected according to this scale.

Fourth.—That the same tube length and cover glass thickness be used in all original corrections of objectives."

To Draw Objects.—It is very important that the appearance of an object should be put upon paper, especially of one which is not permanently mounted. To do this does not require any great amount of skill as the lines which are projected upon paper are merely followed out; but it is necessary that those drawings be made truthful. Nothing should be put down which is not actually seen; neither should any-

thing be omitted. Drawings thus made form a valuable record, not only for the individual, but for others who are following the same line of study.

Camera Lucida—How to Use it.—The camera lucida is the apparatus by means of which drawings are made. There are various forms of these. The methods of attaching to the tubes are also numerous, but a very simple and effective device is that shown

Fig. 19.

in Fig. 19. The mounting is fixed to the cap of the eye-piece by means of a flexible grooved ring.

In this form the pencil point is not plainly visible, and a number of other forms are in use which are

constructed with a special view to avoid this difficulty. They are all, however, considerably more expensive, but should be procured if means will permit.

The procedure of working should be about as follows: Focus upon the object and then incline the body, so that the center of the eye-lens will be 10 inches from the table. To obviate repeated measurements, a standard stick of this length may be used. If the instrument is so low that it will not allow the inclination of the body to an angle of at least 45 degrees when at this distance, it should be placed upon a box; or, if not too high, upon the case of the microscope. Now readjust the mirror and attach the camera lucida from below and place the paper under the instrument; look into the camera lucida from above, being careful that the eye is directly over the center of its opening, and the image will be found to be projected upon the paper. Possibly, and very probably, it will appear faint. This is due to the fact that the paper is almost as highly illuminated as the field. To remedy this defect a cardboard should be placed between the paper and light, so that the former will be shaded; the object will now come out in strong contrast. Take a well pointed pencil and follow the lines in the image. A little difficulty may at first be experienced in seeing both the pencil and image at one time, but after a little practice this is overcome.

It very often occurs that the pencil point can on no condition be seen distinctly, but this is usually due to abnormal sight, with which persons are often afflicted. In these cases, the glasses which are required for reading should also be used in drawing. The difficulty is not experienced in the image, as this can be adjusted to the eye.

Determining the Magnifying Power.—Although a magnifying table may be furnished, this gives the powers merely approximately, as more or less variation occurs in objectives and eye-pieces of the same kind. As it is interesting, and sometimes important, to know the exact magnifying power, a simple method is mentioned. Procure a *stage micrometer*, divided into $\frac{1}{100}$ inch and $\frac{1}{1000}$ inch, and perhaps $\frac{1}{5000}$ inch, or, if prefered, any suitable division in millimetre. Place the micrometer on the stage, focus and incline the microscope, as if for drawing, to within 10 inches from the table and attach the camera lucida; for low powers, $\frac{1}{100}$ divisions may be used; for the higher ones $\frac{1}{1000}$ or more; the division as now projected may be marked upon the paper and then measured off with a rule divided into inches and $\frac{1}{10}$ inches; if, for instance, the $\frac{1}{1000}$ divisions are used, and one division on the paper covered 1 inch on the rule, it is evident that the magnifying power is 1,000 times; if it covered $\frac{2}{10}$, equal to $\frac{1}{5}$, on the rule, it would be 200 diameters, and so on.

Measuring the Size of an Object.—A simple and reliable way of learning the size of an object is

by means of an eye-piece micrometer. As, however, this does not measure the object directly, but only its image, the first part of the process makes it more complicated. However, this portion is usually attended to by the manufacturers. The eye-piece is provided with a slot, into which a micrometer is fitted. A micrometer with the same divisions as the eye-piece micrometer is placed upon the stage and the objective focussed upon it. It is now observed how many of the divisions of the eye-piece micrometer are contained in the magnified division of the stage micrometer, and the resulting figure is placed in the sub-division under the objective. To determine the actual size of an object, this is now placed on the stage and, noting the number of divisions which cover it, these are divided by the number on the card, and the resulting figure gives the actual size. Suppose the figure on the card is 8.0 and the image of the object covers 40 of the spaces which are divided into $\frac{1}{10}$ millimeters, the size of the object would be $\frac{5}{10}$ or $\frac{1}{2}$ millimeter; or expressed in inches, (25.4 mm. equal to 1 inch) about $\frac{1}{50}$.

ADVANCED MANIPULATION.

Dry Adjustable Objectives.—The information which has thus far been given on the manipulation of the microscope may be termed initiatory, as it is supposed (at any rate hoped) to have disclosed some new principles. These are comparatively simple, and with a moderate amount of attention, are easily acquired. It is the intention now to speak of something more complex and to give instruction in the use of higher grade adjustable and immersion objectives. The difficulty of doing this increases with each step of advance, and whether it can be overcome by means of written words, is, perhaps, an open question. However, the writer is certain that if the following instructions are faithfully adhered to, satisfactory results will be gained. The highest attainment must of necessity be the result of perseverance and knowledge of the various properties of an objective, which are given in preceding pages.

It is assumed that a dry objective is used, say a $\frac{1}{6}$ 140 degrees or $\frac{1}{8}$ 135 degrees, and provided with screw-collar adjustment for cover-glass thickness ; it is further assumed that in the first, the variation

between the two first systems (anterior and middle) is attained by means of a rectilinear motion to the middle and posterior system and stationary anterior system, while in the second the conditions are reversed ; the two posterior systems remain stationary while the front is adjusted. Both are arranged with graduations upon the screw-collar and have an index.

In the objectives of the Bausch & Lomb Optical Co., the graduations range from 10 to 35, and as previously stated these numbers indicate the proper points of correction for the respective thickness of cover glass.

In these objectives the open point, *i. e.*, where the objectives are most widely separated, is at 10, thus giving the correction for practically the thinnest covers. As the objective is moved toward the higher numbers the adjustment is closed and gives correction for the thicker covers. In objectives of other manufacture where this system is not adopted, the figures are arbitrary and have no special significance. While the open and closed point can be learned in these by trial, it should be given by the maker before work is attempted with it. Before the objective is attached the adjustment should be closed, as, if this is neglected and the objective has a short working distance, the front lens may come in contact with the cover when it is endeavored to focus on the object.

Probably the best object for studying the effect of the screw-collar adjustment and acquiring skill in determining its best point is again *P. angulatum.*

Place a slide of this upon the stage, and with a low power eye-piece select a diatom which appears to be flat; such a one may usually be found when there are a number on the slide. After the objective has been attached to the nose-piece, focus carefully and observe whether any lines can be seen; if not, grasp the *milled edge* of the *adjustment collar* between the thumb and first finger of the *left* hand, keeping the fingers of the *right* hand upon the micrometer screw, or *vice versa*, if from the outset it was made a habit to use the left hand on the fine adjustment; turn the collar slightly toward its open point, and as this will place the object out of focus, move the fine adjustment correspondingly; continue to turn the collar, little by little, and do not cease to observe closely; also, after each movement, focus above or below the plane of the object, so that this will be indistinct, and look for the lines. Possibly after a little they will begin to appear faintly; but, if not, continue to bring the collar toward the middle. The lines must now soon make their appearance, and, when they do, it will probably be above the plane of the diatom. This is an indication that the objective is approaching its correction for the cover. Now keep the *lines* in focus, while the correction collar is being gradually turned, until the lines and the outline of the diatom lie in one plane; the objective is now said to be *corrected for cover*. Observe which number corresponds to the index, and note this upon paper; again return the collar to its closed point and go through the same

proceeding as carefully as at first. When the best point is again reached, look for the number and see whether it agrees with the first; very likely it does not, which is owing to a want in the faculty of perception, due to a too slight acquaintance with the phenomena. These trials should be repeated until the proper sensitiveness of feeling in making the adjustments is acquired, and until they can be made to correspond with a certainty to at least within two divisions.

Remove the eye-piece and attach one of higher power. It must now, however, be remembered that if there is a considerable difference in the powers there will be a relative difference in their lengths, and that this will cause a difference in the optical length of the tube; this not only will require another adjustment for focus, but will partially destroy the correction as made with the low power. After some practice, the amount of variation may be fixed upon and may be noted for the future; but, to determine it, the same plan as suggested with the low power eye-piece should be followed.

When it is found after repeated trials that sufficient skill has been acquired to bring the collar to within one division, the number and power of the eye-piece should be scratched with a diamond upon the slide or with pen and ink upon the label; thus, if it is found that with a $1\frac{1}{2}$ inch eye-piece the index shows 5, and with a $\frac{1}{2}$ inch eye-piece shows $5\frac{1}{2}$, it should be marked $1\frac{1}{2}$–5 and $\frac{1}{2}$–$5\frac{1}{2}$. In eye-pieces of par-focal construc-

tion it will be unnecessary to mark with different figures, as the correction will be the same for all powers. For future examinations on the same slide, this will facilitate work and give the assurance that the best results are thus gained without further trial.

Mr. Wenham's general rule for obtaining the best correction on objects in general is as follows: "Select any dark speck or opaque portion of the object and bring the outline in perfect focus; then lay the finger on the milled head of the fine adjustment and move it briskly backwards and forwards in both directions from the first position. Observe the expansion of the dark outline of the object, both when within and when without the focus. If the greater expansion or coma is when the object is without the focus or farthest from the objective, the lenses must be placed farther assunder (or opened). If the greater coma is when the object is within the focus, or nearest the objective, the lenses must be brought closer together (or closed). When the objective is in proper adjustment, the expansion of the outline is the same both within and without the focus."

Immersion-Adjustable Objectives.—As was stated before, immersion contact between the objective and cover-glass is made by either water or homogeneous fluid. The fluid should be kept in a small bottle or phial, the cork of which is pierced to receive a small pointed stick or match, and this should project sufficiently so that it will enter the fluid about

$\frac{1}{2}$ inch. The fluid will then always be free from dust and by withdrawing the cork the stick will always carry a drop of fluid with it.

In fixing an immersion objective to the stand, the latter should first be put in an upright position ; the fluid should now be attached to the front lens, but care should be taken not to put on too much ; it should be merely enough to cover the surface. If too much, a portion of it should be removed by allowing it to adhere to the finger. The objective may then be attached to the stand and brought down until the fluid is in contact with the cover ; the stand is now inclined and the objective focused ; if this method is followed there is no danger of flooding the entire cover with fluid, which sometimes may be the means of destroying the object ; neither can the fluid run out from between the two surfaces.

Extreme cleanliness should be observed in all work connected with the microscope, and particularly in the use of immersion objectives. The use of immersion fluid in itself involves a certain amount of inconvenience, but as in many cases it is absolutely necessary, the observance of fixed rules will materially help to overcome some of the disagreeable features. After the work with an immersion objective has been completed, the objective should be removed from the stand, and its front, as well as the slide, should *invariably be cleaned ;* the fluid may be removed by a moist piece of soft linen and then cleaned with a dry piece; chamois skin is not suitable, as it does not absorb the fluid.

Test-Plate.—Almost all microscopists who take an active interest in the capacity of their instruments, supply themselves with a set of *test objects*, of which *P. angulatum* is in most general use, or with a so-called test-plate. These plates consist either of a series of bands of finely ruled lines ranging from 5,000 to the inch to 120,000 to the inch or with a series of diatoms, upon which the markings represent certain divisions of an inch. The one of these which is principally used is made by J. D. Moeller, and consists of a series of 20 diatoms. They are furnished mounted both *dry* and *in balsam*, but the latter is the most common. Below is a table giving the numbers, names of the various diatoms and divisions on their surfaces to $\frac{1}{1000}$ inch A specimen of *Eupodiscus Argus* begins and ends the series :

		Striæ in $\frac{1}{1000}$ of an inch.
1.	Triceratium Favus Ehrbg..........	3.1 to 4.
2.	Pinnularia nobilis Ehrbg...........	11.7 to 14.
3.	Navicula Lyra Ehrbg. var.	14.5 to 18.
4.	Navicula Lyra Ehrbg.......... ...	23. to 30.5
5.	Pinularia interrupta Sm. var........	25.5 to 29.5
6.	Stauroneis Phoenicenteron Ehrbg...	31. to 36.5
7.	Grammatophora marina Sm........	36. to 39.
8.	Pleurosigma balticum Sm..........	32. to 37.
9.	Pleurosigma ȧcuminatum (Kg.) Grun	41. to 46.5
10.	Nitzschia Amphioxys Sm..........	43. to 49.
11.	Pleurosigma angulatum, Sm........	44. to 49.
12.	Grammatophora oceanica Ehrbg = G. subtilissima.................	60. to 67.

13.	Surirella Gemma Ehrbg...........	43.	to 54.
14.	Nitzschia sigmoidea Sm...........	61.	to 64.
15.	Pleurosigma Fasciola Sm. var......	55.	to 58.
16.	Surirella Gemma Ehrbg...........	64.	to 69.
17.	Cymatopleura elliptica Breb........	55.	to 81.
18.	Navicula crassinervis Breb=Frustulia saxonica Rabh...............	78.	to 87.
19.	Nitzschia curvula Sm..............	83.	to 90.
20.	Amphipleura pellucida Kg.........	92.	to 95.

It may be said, and perhaps with truth, that a test-plate does not belong to the necessities of an outfit, but considering that it is a guage, on which the optician usually bases the quality of his objectives, it is valuable to the owner of an objective to be able to determine whether, under his manipulation, the objective will perform as well as is claimed for it ; due consideration must, however, be given to the fact that there is a certain amount of variation among different plates, as is shown in the above table. Outside of this, it is a continual incentive to determine the extreme performance of an objective, and it thus becomes the means of acquiring great manipulative skill, which cannot be underrated. The writer is in a position to know that there is great need of this; innumerable cases have come to his notice where several objectives of the same kind and equal quality gave unequal results in different hands, and would be highly eulogized by the possessor of one and condemned by that of another.

Immersion Objectives on Test Plate.—To determine the highest capacity on test objects, ordinary daylight is hardly sufficient; moderate sunlight or good lamp-light is best suited, but the latter, from the fact that it is always at hand, is preferable. For the purpose of explanation, we will assume that a flat wick lamp and a $\frac{1}{8}$, $\frac{1}{10}$ or $\frac{1}{12}$ homogeneous immersion objective is used. If the right hand is used on the micrometer screw, place the lamp at the right side of the instrument, about 10 inches from it, with the *edge* of the flame turned toward the mirror.

The test plate may now be placed upon the stage, and as the diatoms in balsam are very transparent, and therefore very difficult to find, a lower power objective may be used as a finder; bring No. 1, or *Triceratium Favus*, in the center of the field, and after the objective has been removed, attach the immersion objective in the manner prescribed; the adjustment collar may be placed at zero, as this is about the correct point for standard length of tube. Get the best possible illumination with the mirror at the central point and move the test plate from diatom to diatom until it reaches No. 11, *P. angulatum*, but observe closely the structure of each one as it comes into the field. Next see whether the objective is corrected; if the lines and outlines, or middle rib, do not appear to be in one plane, adjust the collar until they are, and then continue the advance toward the higher numbers until one is reached on which no lines can be

seen. Swing the mirror-bar to an obliquity of 20 degrees, and, readjusting the mirror, observe the effect. It is very probable that the lines will show, and, if so, continue the advance; if they do not, give 10 degrees or 20 degrees more obliquity, and after the structure comes out, again go forward. A point may thus be reached, where with the greatest obliquity which can be given and with the best possible illumination, the objective seems to have come to the limit of its performance. From the claims which have been made for it, it ought to do better. What is the cause of failure? Possibly the mirror is not correctly focused, or the adjustment collar may not be correct for oblique light; perhaps the eye-piece does not give sufficient magnifying power to distinguish the striæ. It may be any one of these causes or all combined. As to the eye-piece, the manipulator must remember the amount of separation of lines in the last object which was resolved, and from the gradation in the coarser specimens must judge whether the power is sufficient; it should be added that for any over No. 14 and under No. 18 a $\frac{3}{4}$ inch eye-piece should be used, and for those above No. 18 a power of $\frac{1}{2}$ inch will probably be necessary, provided a $\frac{1}{8}$ or $\frac{1}{10}$ objective is used. After this condition has been complied with, look to the correction collar of the objective; to obtain the highest results it very often occurs that a different adjustment is required for oblique light from that for central light. Note the number at which it stands, and then work it back and forth,

watching carefully for results. If this has no influence, return it to its number or to a point where the outline of the object appears most sharp. Now look to the illumination ; vary the distance of the mirror to the object, and, if it conflicts with the stage or does not give the desired results, vary the distance of the lamp to the instrument and watch the effect of the change through the tube. A great change in the illuminating power can thus be produced ; the light is best when it covers the least space, as it is then most intense. The light may be quickly adjusted by throwing it upon a point on the slide in the opening of the stage and watching it there. If neither of these changes give any improvement, recourse must be had to another expedient. Place a bull's-eye between the lamp and mirror with the plane side to the former, and close to it so that the light is thrown on the latter. It should be properly concentrated so that the circle of light will not be larger than the mirror, which can be determined by placing the hand or a piece of paper back of it. Adjust when necessary by moving the lamp or bull's eye. Keep it a little below the line of the face of the stage, so that the light will not strike it on its upper and as little as possible on its lower surface ; if the light from the bull's-eye directly reaches the object, it destroys the effect of the oblique illumination. Great care should be given to this point, as it is very important.

If all of these suggestions have been followed, a great difference will undoubtedly be noticed in the

performance of the objective; but if it still does not come up to the standard, patience must not be lost. The slightest change in the mirror, bull's-eye, or lamp, a touch to the correction collar or micrometer screw is sometimes followed by astonishing results. The beginner should sit down with the expectation that he will fail at the first trial. At each succeeding trial he can easily notice his improvement in manipulation and the gain of corresponding results. He should be able to bring the performance of the objective up to the claims made for it, if it has come from the hands of a reliable optician, and should not rest until this is accomplished.

The writer has often recommended sunlight with generally successful results where ordinary means of illumination have failed. The light is of course intense, and great care will have to be used to modify it by properly using the mirror, but success is often attained and then creates confidence. It is, however, only recommended for this purpose and not for general use.

To the histologist it may seem strange that the writer has thus far only spoken of working with objectives on diatoms. This, however, was done advisedly. They are thin, and therefore as suitable as a thin section and far more preferable than a thick one. Their form and structure are easily recognizable, and there is very little variation among those of the same kind; therefore, rules laid down regarding them are generally good. It is conceded by advanced

workers that the time spent over diatoms for the purpose of studying objectives is well applied, and the most expert manipulators have acquired their experience in this manner. An objective which works well on diatoms works equally well on other objects, and therefore the manipulative skill which has been attained on the former is as well applied on the latter. At the outset work may be done on other objects than diatoms, and where ordinary working objectives, such as a Student 1 inch and $\frac{1}{4}$ inch, or $\frac{3}{4}$ and $\frac{1}{5}$ inch comprise the outfit, the road to good manipulation may be as short as with diatoms. The conditions in both cases remain the same; but it must be cautioned that, if histological preparations be used, only such be selected as are reliable. A poor specimen is perhaps as bad as none at all; an abnormally thick one obstructs light, makes it impossible for the objective to penetrate through the various layers, and leaves the impression that the latter is defective.

Photo-Micrography.—This subject does not properly belong within the scope of this book, but there are some points connected with it which may be of value to mention.

Any person desiring to do this work should endeavor to obtain some experience in ordinary photography and proper developing. First, as good results in photography with the microscope are difficult to obtain, even with previous experience, a book devoted to this purpose should be well studied before attempting to obtain results. The beginner in this

direction will find it somewhat difficult to make a proper selection of apparatus. The most inexpensive is to use the ordinary microscope in a horizontal position and attach to an ordinary camera, in which care will have to be observed to see that the ground glass is at exactly right angles to the optical axis. As a rule the ordinary view camera is not of sufficient accuracy to use without specially adapting it to this purpose, and the main difficulty lies in the lack of coincidence of the ground glass with the film side of the plate. Plates in themselves are irregular and the shoulders on which they rest should at any rate coincide. The best plan to determine this is by placing a straight edge on the frame of the ground glass, interpose a wooden wedge between it and the ground surface of the glass and mark the point of contact by a pencil mark. Follow the same procedure with the plate-holder containing the plate. The variation can in this manner be seen with a nicety and proper correction easily made. The writer recommends that this test be made with each plate, so that this is always in exact coincidence.

Where means will permit, the best plan is no doubt to obtain an apparatus which is complete in itself and is used for this purpose only. The Atwood, as an inexpensive, and the Rafter as complete in every direction, may be recommended.

Whether an eye-piece should be used or not is a matter of controversy. Both methods are followed with good success. By the use of the eye-piece the

microscope is brought to its normal condition and the projection of the image into the camera carries with it the faults of the eye-pieces, and these increase with the length of the camera. Without the use of the eye-piece the spherical and chromatic correction of the objectives is disturbed in proportion as the plate is distant from the standard tube length and will destroy the definition of a high power objective unless compensation can be made by means of collar correction.

The plan followed by a well-known worker is to take a negative at the end of the tube without the eye-piece, where the proper corrections are obtained, and from this enlarge to any suitable size.

The amplifier should be used where the eye-piece is not employed, as by its proper adjustment the objective may be brought to its normal condition. This is the principle involved in the Rafter camera. Its use is therefore not that of an amplifier but that of a corrector.

The question of suitable objectives is one of con-considerable importance. The ordinary microscope objective is not constructed with a view to photographic work, and unless nicely corrected there will be a lack of coincidence between the visual and chemical rays, *i. e.* between the image which is seen on the ground glass and that which is photographically produced. For all this, practical experience has shown that a number of Bausch & Lomb Optical Co. objectives are well suited to this work. For low powers the Student and Professional series may be success-

fully used. For medium powers special objectives are constructed, and for high powers any of the homogeneous immersion may be successfully employed.

Some favor the apochromatic objectives as these are specially constructed with a view to using them in this work, but their considerable higher prices plelude their use in many cases.

TO SELECT A MICROSCOPE.

When a person has concluded to obtain a microscope, a suitable selection is a matter of considerable importance to him. The varieties are innumerable, prices without end, all sorts of claims made for them. It is, therefore, easily explained why this chapter should be an important one in a manual of this kind, and yet difficult to treat satisfactorily.

The variety of special lines of investigation involves nearly as great a variety of requirements. The amount of money to be expended; what shall be the stand; what the objectives; shall the entire outfit be purchased at one time or little by little, are all questions of paramount importance which the writer does not expect to solve, but hopes to give sufficient information that a more intelligent selection may be made than might probably be done otherwise.

Stands.—Starting out with the assumption that there are two classes of instruments to select from, the long and short tube, the first decision to reach will be this point. In a general way it may be said that there are no optical advantages in either, but whichever is adopted must be retained.

The principal consideration is whether the instrument is to be used in an upright or inclined position. If the former, the short tube is most usually selected, as it can be used comfortably on a table of ordinary height. This one objection which might be and often is raised against the long tube is easily overcome by the friends of the latter providing a suitable table for the same. As the instrument is used in the upright position only in a few special lines of study, it is really only of weight in this direction, as the instrument may be inclined to the most comfortable point, and when so is more comfortable than the upright position.

The joint for inclination of arm is generally conceded to be an advantage. While it may be the case that many of the upright instruments are in use in Europe, there are very few used in this country, and the preponderence of instruments shown in catalogues of foreign makers, would indicate the same tendency.

Almost all instruments for reliable work are provided with both fine and coarse adjustment. They are both necessary, the only question being whether the latter shall be by the sliding tube or rack and pinion. The former, while perhaps having the advantage of admitting a more speedy change of objectives, has a decided disadvantage in the hands of the students in endangering objectives and preparations. Further than this, it is almost impossible for the maker to center the nose-piece with the tube, so that a change of objectives usually loses an object out of

the field, and requires that it be looked for anew with each change. In the rack and pinion the nose-piece has an unvarying relation to the tube, and is not liable to this difficulty, and offers a steady and agreeable adjustment. The advantages of the rack and pinion seem to be generally appreciated in this country, for there are few instruments sold and used without it.

Whether an instrument shall be of japanned iron or lacquered brass is probably largely determined by the amount of money to be expended. As far as the intrinsic suitability of the metals is concerned, there is no difference. Brass, however, offers the maker a better opportunity for displaying his mechanical skill, and while it is no doubt true that many highly finished instruments are of poor workmanship in their working parts, it is also a fact that a well made instrument is always nicely finished.

The size of instrument is worthy of consideration. If an instrument is to remain stationary in a practitioner's office or laboratory, it may be large without being cumbersome. If, however, it is intended to be carried about, it should be of the smaller and more contracted style.

Another important consideration is the space between the stage and base, or table. While it is advisible to have the stage low on account of the convenience in manipulating a slide, there should still be sufficient space for the convenient attachment of substage accessories. As a rule the American pattern of

instruments provide more room between the stage and base on the lower side and stage and tube on the upper, than do the Continental.

As stated previously, a variety of stages are offered on instruments of similar construction. The plain flat stage while preferred to some, offers no advantages over the ordinary round one, unless specially made for examining specimens on larger slides than the standard 3 by 1 inch. Some claim advantages for a smaller stage than the length of the slide, so that this, projecting, admits of the slide being grasped and swung around the optical axis. These advantages, however, are not generally appreciated, and even if so, are offset by the drawback that in moving the slide it is apt to be tilted.

Spring clips are usually of similar construction, although varying in detail and curves. Properly constructed clips should have such thickness of metal and be so bent as to allow specimens to be brought under them without resistence and keep them properly in place without too much pressure and consequent friction.

A glass-stage and slide-carrier may be considered a good investment, as it admits of the convenient manipulation of the slide without the grating feeling which usually accompanies the direct movement of the slide on the stage.

Where systematic examinations of a specimen are to be made, a mechanical stage will be found a great convenience and in petrographical work is almost a necessity.

A sub-stage may, in a general way, be said to be preferable when it is adjustable, particularly in the use of a condenser. It is absolutely necessary to adjust the condenser for different objectives and this must be done so nicely that a sliding movement is hardly sufficient. In biological work the condenser is at the present day a necessity, and this is now constructed as a separate attachment with all the necessary adjustments, in which the rack and pinion is deemed of first importance.

Objectives and Eye-Pieces.—It is hoped that the information given of the various qualities in an objective will aid to make a suitable selection of the optical parts. As the stands have been classified in long and short standard tubes, the first quality to look for is, after the stand has been selected, their suitability to it.

As will have been seen under the proper head, a variety of powers is obtained by a suitable combination of eye-pieces and objectives, and while power alone can be obtained by increasing the power of the eye-piece, it is not advantageous to do so. For ordinary work no higher eye-piece than a ¾ should be used. In catalogues many outfits are made up of one eye-piece and two objectives, but this is only for the purpose of reducing price to a minimum. It is always advisable, when means will permit, to select two eye-pieces, preferably the 2 inch and 1 inch, and insist they be par-focal, as this will be found extremely convenient and will not disturb the optical standard

length. If for any work $\frac{1}{2}$ inch or higher powers are desired, the solid eye-pieces may be recommended. The periscopic are advantageous for micrometric tests and other work where a large or flat field is desirable.

For student's and practitioner's use, the outfits as made up in catalogues are usually sufficient, except, as above recommended, where but one eye-piece is given, it is well to select two where means will permit.

Referring particularly to the catalogue of the Bausch & Lomb Optical Co., we will state the purpose for which they were intended

Biological—Laboratory, student and professional use.

Large Biological--Advanced laboratory, student and professional use.

Harvard—Laboratory, student and professional use.

Model—Laboratory, student, professional and amateur use.

Physician—Professional.

Investigator—Student, professional and amateur use.

Universal—Student, professional and amateur use.

Concentric—Professional and amateur use.

Professional — Advanced laboratory, professional and amateur use.

For ordinary professional use, including urinary examinations, the $\frac{3}{4}$ inch 27 degrees and $\frac{1}{5}$ inch 110 degrees objectives will be found sufficient. For bacteriological examinations a higher power, such as a $\frac{1}{12}$ inch homogeneous immersion, objective will be necessary.

If means will permit, an investment in the $\frac{3}{4}$ inch 40 degrees and $\frac{1}{5}$ inch 130 degrees, or $\frac{3}{4}$ inch 40 degress and $\frac{1}{6}$ inch 140 degrees, will be well applied. This latter is in very general use and may be highly recommended.

In botanical work a lower power than those mentioned, such as a 2 inch 12 degrees or, preferably, 2 inch 15 degrees, will be necessary.

For amateur use the ordinary outfit of $\frac{3}{4}$ inch 27 degrees and $\frac{1}{5}$ inch 110 degrees with the addition of a 2 inch, preferably of the better grades, will do. If the examination of diatoms will be followed, the $\frac{1}{6}$ inch 140 degrees and $\frac{1}{12}$ inch homogeneous immersion, will probably be required.

For the student, the $\frac{2}{3}$ inch 28 degrees and $\frac{1}{6}$ inch 116 degrees, or $\frac{3}{4}$ inch 27 degrees and $\frac{1}{5}$ inch 110 degrees objectives, will ordinarily be ample.

Although from an optical standpoint it is true that objectives give more detail as they increase in their angular apertures, it will have been seen that the highest class of objectives is not always recommended. A great portion of everyday work does not require this maximum of optical results, and can be accomplished completely and with comfort with objectives of comparatively low aperture. Some years ago microscopists were divided into two classes, the new school of wide aperture, and the old school of narrow aperture. The state of affairs existing then has happily changed, concessions having been gradually made, so that now the advantages of both classes of objectives

are appreciated and there are few microscopists of standing who would recommend only one or the other kind.

In these days of competition, prices alone are too often made the object of inducement, without any reference to quality. Be distrustful of all such objectives, and if contemplating their purchase, always reserve the right of having them examined by an expert. Have a distrust especially of all "nameless" objectives. It is safe to assume that if the maker can not attach his name he is doubtful of their superiority. Any maker of responsibility will say without hesitation that he can produce objectives at less than one-half their present cost, if he had the assurance that they would be accepted as first put together, as the cost of merely making and mounting lenses is considerably less than the cost of making proper corrections. In this case, however, they would be of varying and inferior quality.

It is sometimes found that dealers offer the same objectives of different quality at different prices. Too great care cannot be observed in such cases, as the very fact of the admission of a difference in quality indicates that they are made by an unreliable maker. This mode of offering objectives was in vogue many years ago when the principles of optics and facilities for making were limited, and when a higher price was asked for those which might be termed a happy combination. There is no excuse, however, at the present day, for anything of this kind, because every con-

scientious optician has his standard for every objective which is his guide.

In purchasing a microscope a beginner may be easily misled by the enticing appearance of an object, which may be due not so much to the instrument as to the object itself, and if the optical parts are inferior, it will require but a short experience to become convinced of it—usually as soon as a comparison can be made with reliable work. The investment in one of these objectives is not only a source of disappointment, but usually proves to be a pecuniary loss, as it is usually followed by a fresh outlay in responsible work.

It is of ordinary occurrence that such objectives as have just been spoken of are sent to the writer's firm with the request to examine them and rectify the faults; but an examination almost invariably proves that the cost of doing this is considerably greater than purchasing a new objective of the same power, and it would not even then be equal to the latter.

Accessories.—As has been stated before, one of the most useful accessories to the stand is the glass-stage and slide-carrier.

Another accessory which is in equal demand, and deservedly so, is the double nose-piece. By means of it two objectives may be kept permanently attached to the microscope, avoiding any loss of time from changes. When properly fitted, an immediate change can be made with the additional advantage of having

the objectives centered and adjusted for focus, or nearly so, which is a very decided consideration.

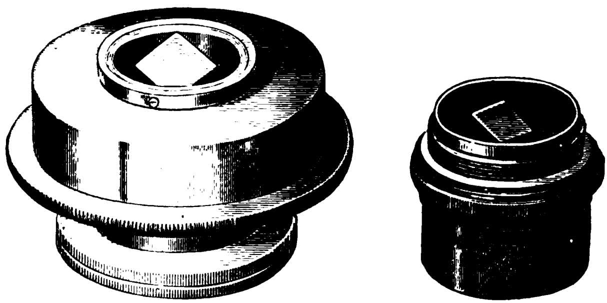
Fig. 20.

The same advantage holds good for the triple nose-piece in the case of three objectives, or the quadruple nose-piece for four. In these it is usually not convenient to arrange the objectives to correspond in focus on account of the considerable difference in focal distance.

The next in order of demand is the Abbe condenser in its various forms of mounting. While some able microscopists hold that the proper use of the mirror will satisfy every demand, and that unless properly used the condenser is disadvantageous instead of beneficial, it is unquestionably true that it will give results which the mirror alone can not properly give. Furthermore it enables results to be obtained easily, which would require careful and long experience by the mirror. The writer is in the best position to know from the many inquiries received from professional men, on how to increase the light, that for this purpose and the better distinguishment

of structure alone, it may be considered a boon. Biological work cannot be successfully prosecuted without it.

The polariscope is absolutety requisite in petrographical work, and in the examination of crystals in connection with a selenite, it is an excellent appar-



Figs. 21.

atus in the hands of professional and amateur for instruction and amusement, bringing out wonderful coloration of many objects. Although the microscope is really an instrument for scientific research, it does not suffer by being used as a means for recreation and pleasure, and in this direction no accessory will aid more than a polariscope. Three selenites may be used with it, giving the following combination of colors: Blue and yellow, red and green, purple and green. The polarizer is always arranged to resolve.

The bull's-eye condenser is mainly intended for the illumination of opaque objects, and although modern instruments have their mirrors so hung that they may

be brought above the stage for this same purpose, the results are not so satisfactory. The bull's-eye may be used by interposing it between the mirror and source of light, and with a very material increase in its volume and easy regulation.

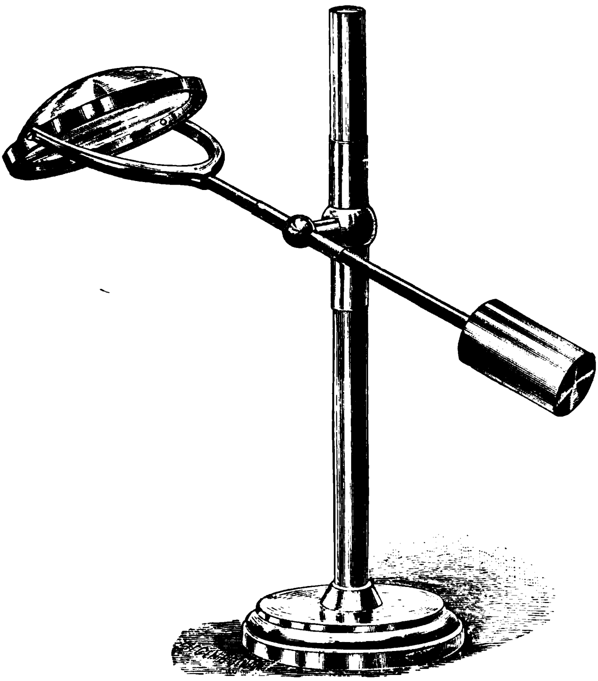


Fig. 22.

The Mechanical Stage, Micrometer, Camera Lucida and other accessories have been treated separately, and will therefore require no farther enumeration.

SUB-STAGE ILLUMINATION.

While the objective and its proper application and use will always be the most important part of the microscope, the proper illumination of objects is second in importance.

Mirror.—As has been stated, the mirror alone offers an excellent means of properly displaying an object when intelligently used, for most of the low and medium power objectives. On account of the limited capacity of old objectives, efforts have been made for the past fifty years to increase their utility by the aid of lenses below the object. This was mainly in the way of increase of intensity which at the present day is not of so much consequence, as our sources of illumination and light conveying capacity of objectives is so much greater.

Abbe Condenser.—The history of sub-stage condensers is very unique and interesting, and shows how from having been the subject of no end of condemnation it is now the reverse. From single lenses, compound, non-achromatic and achromatic, the use of eye-pieces and objectives with any number of

devices for regulating the light, the generally accepted forms are those devised by Prof. Abbe. One of them with a numerical aperture of 1.20 consists of a combination of two lenses, and the other with an aperture of 1.42, of three lenses. These condensers are applied to microscopes by each maker in a different way, each seeking in his own manner to provide means for controlling the volume or angle of light as well as to obtain any degree of obliquity. To bring the condenser to the minimum of cost, the Bausch & Lomb Optical Co. quote both condensers in plain adapters, giving the entire condensing capacity which may be varied only by varying their distance from the object. In this form they are incomplete, however, as their effectiveness depends upon the nicety with which the light can be controlled. The most

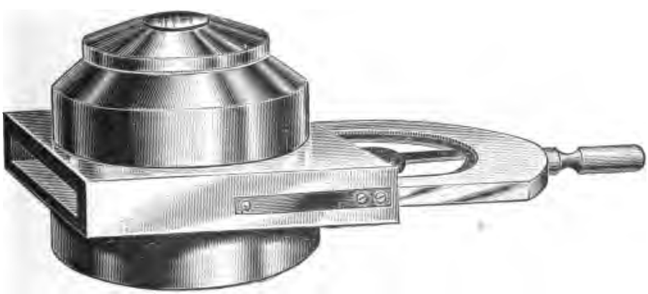


Fig. 23.

simple device for this purpose consists of the mounting with a slide in which various diaphragms with central stops and openings may be dropped into it, and

brought into the optical axis or passed diametrically across the condenser, giving different degrees of obliquity.

The vertical adjustment is obtained by sliding in the sub-stage.

The best form is that which is provided with an Iris diaphragm, which, when open, gives full volume of light but may be reduced to nearly a pin-hole opening. Then an addition which carries it laterally under the condenser by rack and pinion, and another rack and pinion for vertical adjustment either on the sub-stage bar or as part of the condenser. As the successful utilization of the condenser depends upon the nicety with which the various adjustments can be accomplished, they can be none too complete.

Centering.—In using the condenser the first condition is the proper centering. For this purpose the pin-hole cap should be placed over the top and focused upon by means of a medium power objective. The condenser should be fixed so that the opening will be in the center of the field. For objectives under 1.0 numerical aperture it is not necessary to bring the condenser in immersion contact with the slide; for objectives of wider aper-ture, however, it should be done by first placing a drop of immersion fluid on the top lens and then placing the slide in position on the stage, raise the condenser until the fluid comes in contact with the lower surface.

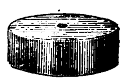


Fig. 24.

It might be said here in parenthesis that the *plane mirror should always be used* with the condenser.

To Focus Condenser.—An excellent plan for focusing the condenser is to do so by means of a low power objective, $\frac{3}{4}$ to 3 inch, in the microscope tube. After the slide is in contact with the condenser or when not used with immersion and close to it, focus the objective on the object. Now adjust the condenser until the image which it projects of the source of light is coincident with it. In the case of a lamp, the flame will be projected. Daylight offers no tangible image, and while the image of the window-frame is not really quite correct, it is, in most cases, sufficiently close for all practical purposes.

Intensity of Light.—To say what amount of light shall be used is very difficult on account of the variety of specimens which involve different conditions. While it is claimed that on stained bacteria the best plan is to use the full volume of illumination and thus differentiate the objects, it is certainly detrimental to do so on many histological specimens, diatoms and others, as they would be drowned in light to such an extent as to be in some cases indistinguishable. The safest plan will be to use the minimum of light and gradually increase until the point for proper observation is reached. It will also be a good plan not to use more light than is needed to accomplish the purpose desired.

To obtain oblique illumination, reduce the aperture to $\frac{1}{8}$ inch and gradually bring the opening out of the

center up to the limit of aperture of the objective. When beyond this, the object will appear illuminated on a dark ground. The lateral movement should be at right angles to the striæ which it is desired to see, and if this is not known, either the object should be revolved or the mounting of the condenser. It will sometimes be found advantageous to reduce or enlarge the opening.

CARE OF A MICROSCOPE.

Besides acquiring the ability to properly use an instrument with its accessories, it is important to know how to keep it in the best working condition. It may be said without reserve that an instrument properly made at the outset and judiciously used should hardly show any signs of wear either in appearance or in its working parts, even after the most protracted use; and further than this, every good instrument should have a provision for taking up lost motion, if there is a likelihood that this may occur in any of the parts.

Especial care should be given to the optical parts, in fact such care, that they will remain in as good condition as when first received. Accidental injury may of course occur to them, but if a systematic manner of working is followed and a special receptacle for each part is provided, this may usually be avoided. The following rules refer mainly to the instruments manufactured by the Bausch & Lomb Optical Co., but are applicable to instruments in general.

To Take Care of a Stand.—One of the first rules should be to keep the instrument *free from dust.* This may be done in a manner formerly prescribed. If dust settles on any part of the instrument, remove it first with a camel's hair brush, and then wipe carefully with a chamois skin, *with* the grain of the finish and not across it, as in the latter case it is likely to cause scratches. Keep the working and sliding parts absolutely free from dust, as this grinds and will thus soon cause play.

Use no alcohol on any part of the instrument, as it will remove the lacquer. As the latter is for the purpose of preventing oxydation of the metals, it is important to observe this rule.

In using the draw-tube impart a spiral motion. In instruments which have no cloth lining, a straight up and down movement should be employed, as the tube will otherwise become scratched.

If it becomes necessary to lubricate any of the parts, use a slight quantity of soft tallow or good clock oil.

In an instrument which is in constant use, it sometime occurs that the pinion works loose and occasionally to such an extent that the body drops of its own weight. Tightening screws are provided to take up the play—in the Professional, American Concentric, Universal, Physician, Biological and Library Microscopes these are in the back of the pinion. In the Investigator, Model and Family Microscopes, they are seen in the slide by removing the body.

In using a screw-driver, grind its two large surfaces so that they are parallel and not wedge-shape, and so it will exactly fit in the slot of the screw-head.

In inclining the stand *always* grasp it at the arm and *never* at the tube, as in the latter case it may loosen the slide or tear off some of the parts.

When repairs or alterations are necessary, always have these made by the manufacturers; they can, from the system of duplicated parts, not only do it cheapest, but best.

To Take Care of Objectives and Eye-Pieces.—It is as necessary to keep these *free from dust* as the stand, in fact even greater cleanliness should be observed. When indistinct, dark specks show in the field, the cause may usually be looked for in the field-lens, although sometimes in the eye-lens also. The dust may be removed by a camel's-hair brush, but when this is not sufficient use a well washed piece of linen, such as an old handkerchief. From its fine texture chamois skin is desirable, but as it is fatty it should never be used until after it has been well washed.

The same method applies to cleaning objectives. Clean an immersion objective invariably after it has been used, first by removing the fluid by a moist linen and then by using a dry piece.

Keep the objectives especially in such a place where they are not subject to extreme and sudden changes of temperature, as the unequal expansion

and contraction of glass and metal may cause the cement between the lenses to crack. Also keep them from direct sunlight.

Screw them into the nose-piece and unscrew, by grasping the milled edge.

Avoid any violent contact of the front lens with the cover-glass. Usually the latter suffers, but it is as liable to occur to the former.

Above all, it should be made a rule that no one but the owner handle the microscope and accessories. One person may be expert in the manipulation of one instrument and still find it difficult to work with another. The fine adjustment particularly causes the greatest difficulty as in some instruments it corresponds with the movement of the micrometer screw, while in others it is contrary and thus the objective as well as object are endangered.

APPENDIX.

(Reprinted from "The Microscope," Jan., 1885.)

CONSIDERATIONS IN TESTING OBJECTIVES.

EDWARD BAUSCH.

There is a laudable desire in almost all persons possessing a microscope to become intimately acquainted with it, and for this purpose it is not only necessary to learn the use of its mechanical parts, but to understand its optical capacity, which is considerably more difficult, and which involves more considerations than would appear on first thought.

With all the care which may be bestowed upon objectives, they are, to a certain extent, works of chance, and depend upon the optician's judgment, industry and skill, and upon the variations in glass, for their excellence and uniformity. These conditions are often so varying that in the case of sev-

eral objectives of the same formula, made at the same time there will be such great differences that it can hardly be conceived on the first examination, that they were to be similar. It is at this point especially necessary to detect the errors, to determine their cause and apply the remedy, and to do this properly often involves an inconceivable amount of work, and in many cases the final results are reached at a pecuniary loss. There are certain fixed tests for each kind of objective, and to the best of my knowledge all reputable opticians bring each objective up to its standard before allowing it to pass their hands, irrespective of the cost of doing so. This must of necessity be so, if only out of business consideration, and not for a love of each production, for it is evident that a well-earned reputation would soon lose its pre-eminence, and would acquire one for unreliable or poor work, if on comparison, objectives of the same kind would show a marked difference. There is sometimes a fortunate combination of circumstances which makes a certain objective better than its fellows, but this is a rare exception, and is positive evidence that the exact requirements of the formula have been complied with. As a rule, therefore, I believe that the opticians' claim may be relied upon, and where the results in the hands of the microscopist do not correspond with them, the cause may usually be looked for in the lack of experience in manipulation or in conditions, which differ from those under which the objective was completed. The belief, which I am aware is

extant, that there are great differences in objectives purporting to be similar, is, in my opinion, not justified, at any rate in the productions of those men who, by general acknowledgement, are at the head of their profession. I admit that, as in everything which depends upon human skill, there is, strictly speaking, no absolute uniformity, but also claim, that with few exceptions, the differences are so slight, that anything but the most expert manipulation cannot detect them.

It therefore appears to the writer that any information which will tend to improve the knowledge of testing objectives will not only prove beneficial to the microscopist, but will prove advantageous to the optician, in that his work will receive a fair trial, based upon a knowledge of the principles involved, and that he may be convinced that all his work which deserves commendation will be the better appreciated. The following points are by no means new, but are often lost sight of in making tests. The writer will speak of medium and high power objectives only, as the deleterious influences are most noticable in these, but they apply as well to the lower powers though in a less degree.

The part of the instrument which has a strong bearing in the performance of the objective is the mirror. It should be adjustable on the mirror-bar, so that it can be accommodated to the variations in distance of the source of light from the instrument. When parallel rays are used, as with light from the sun or clouds, its distance from the object should be

decreased and increased when lamp-light is used. It should be exacted that the focus of the concave mirror be within the limits of its adjustment. The serious disadvantage of its incorrectness in this respect can easily be seen by taking, for instance, a $\frac{1}{5}$ objective which will resolve *P. angulatum* nicely with central light, when the mirror is exactly focused. By moving the latter out of focus it will be seen that the objective loses in performance, and if this is carried sufficiently far it will arrive at a point where the objective will cease to show any lines. The effect will be the same on any other object, and is caused by the lack of proper concentration of light on the slide. When oblique light is used, unless the diaphragm moves with the mirror, it should be removed, as the advantage of obliquity is diminished or destroyed by the loss of light.

The cover-glass exerts probably the greatest influence in testing as well as in general work. This should be used of a thickness which corresponds to that to which the objective (if non-adjustable) was originally corrected. If thicker or thinner covers be used, the objective will be spherically over or under corrected, and will have to be moved correspondingly above or below the plane (outline) of the object to distinguish its structure, if the variation is considerable the difference between the two planes will be so great that it will cease to show any structure, and it may then be said to be lacking in defining power, although in reality it possesses it but is not

properly used. Generally speaking the objective may be said to be spherically corrected when it gives the best defined image; that is, when the outline and internal structure of an object of extreme thinness appear in one plane. When, after the objective has been focused on the outline of the object, it is necessary to increase the distance to focus on the structure, it is evidence that the objective is spherically over-corrected and that the cover is too thick; in adjustable objectives the correction collar must be brought to its closing point, which means that the lenses are brought in closer contact. When the objective must be focused to a point beyond the outline of the object to see its structure--that is, brought closer to the cover-glass—it proves that this is too thin, and is then said to be spherically under corrected; to give the proper adjustment in an adjustable objective in this case the adjustment is opened—the lenses are separated. It requires a certain amount of study to distinguish these phenomena, and although it can be done in well prepared specimens, I know of none better than coarsely marked diatoms, such as *P. angulatum*.

Although I am aware that many eminent microscopists do not favor adjustable objectives for every day work, I must confess that I fail to see the force of their arguments. From the foregoing it will be seen that unless the cover-glasses are of a thickness corresponding to that which was originally used, the objective may be made to do imperfectly what is in its

power to do well, and when pressed to its full capacity may and is likely to fail. It must be remembered that cover-glasses of the same number are not of the same thickness. The selection of those of proper thickness is expensive and tedious, whereas the knowledge of correcting the objective is easily acquired, and in the latter case it is in the manipulator's power to command the highest performance of which the objective is capable; further than this, it has the advantage that it may be used as a non-adjustable objective if desired. When homogeneous immersion objectives were first introduced they were mounted in fixed settings, as it was expected that the thickness of the cover-glass would not affect the correction; although this assumption was correct, it was found that even in these it was necessary. How much more then, is it required in dry or water immersion objectives?

Another factor in the disturbing influences is the variation in length of tube; the deleterious results are similar to those with varying cover-glasses. Objectives are usually adjusted to 8½ or 9 inches length of tube, and although this in itself is a fixed standard, it usually becomes variable by changing objectives and eye-pieces. That this is so in objectives is patent, and that it is so in eye-pieces can easily be determined by making a change in powers, when it will be found that a change in focus is required. By decreasing the length of tube the objective will appear to be spherically under-corrected and *vice versa* when it is increased,

so that it is apparent that by the use of the draw-tube the effect of the cover-glass may be partially neutralized; for instance, when by the use of a thin cover the objective is spherically under-corrected, it may, to a certain extent, be corrected by causing a corresponding over-correction in the tube by increasing its length. The use of the draw-tube for the purpose of changing the amplification or for the matter of convenience can hardly be commended, except in cases where adjustable objectives are used.

Considerable also depends upon the perfection of the eye-piece. I believe that, as a rule, too little care is devoted to it; at any rate, it is certain that while any Huyghenian eye-piece for a telescope can be used on a microscope, very few which have been made for this can be used on a telescope; and while it is true that no such perfection may be required in the former, it leaves such an indefinite range that it may become difficult to place a limit for the perfect and imperfect. In all work, and especially in testing, it should be seen that the eye-lens, as well as the field-lens, are perfectly clean.

Among the absolutely necessary conditions in judging of the quality of an objective are perfect specimens, especially if they are sections. A thick object obstructs the light and generally makes it necessary to go through so many layers or planes that it is difficult to get any one distinct; the impression may thus easily be given that the objective is at fault. The difference between two objects of the same nature

may be so great that, while with one the objective may be condemned as imperfect, it may with the other appear to be of extraordinary excellence.

In conclusion, I will say that there may be other conditions which may influence the performance of a lens, and to acquire the power of eliminating them requires considerable experience. When an objective does not correspond with the claims of the optician, judgment should not be passed upon it until after repeated trials have been made, in all of which the above points should not be lost sight of.

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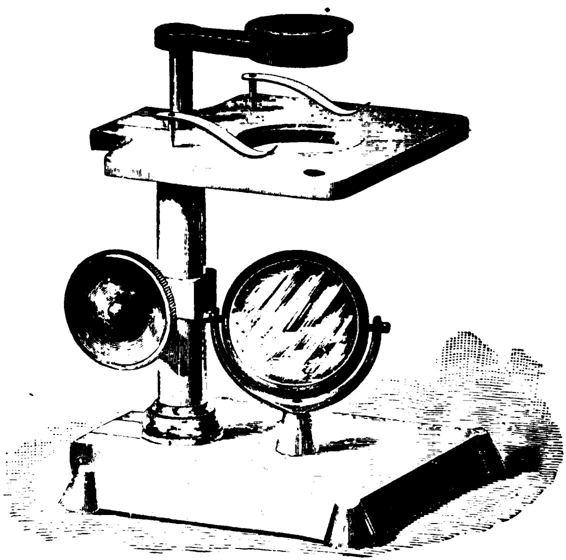

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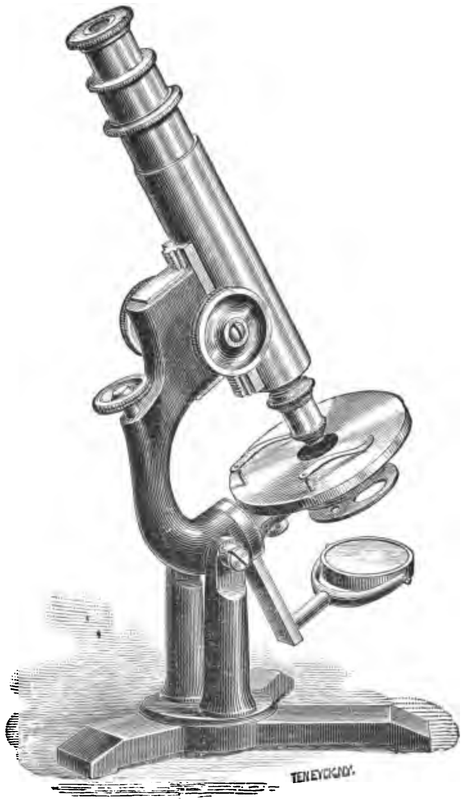

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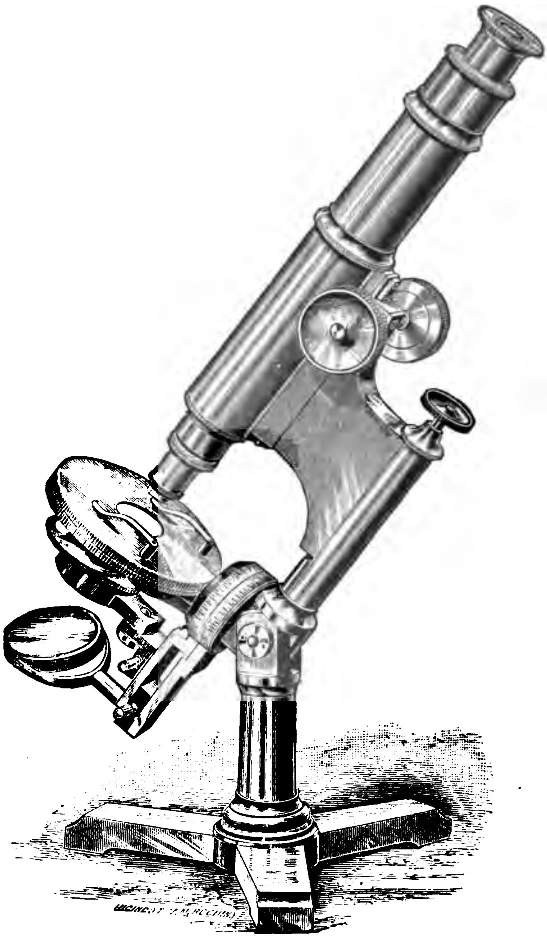

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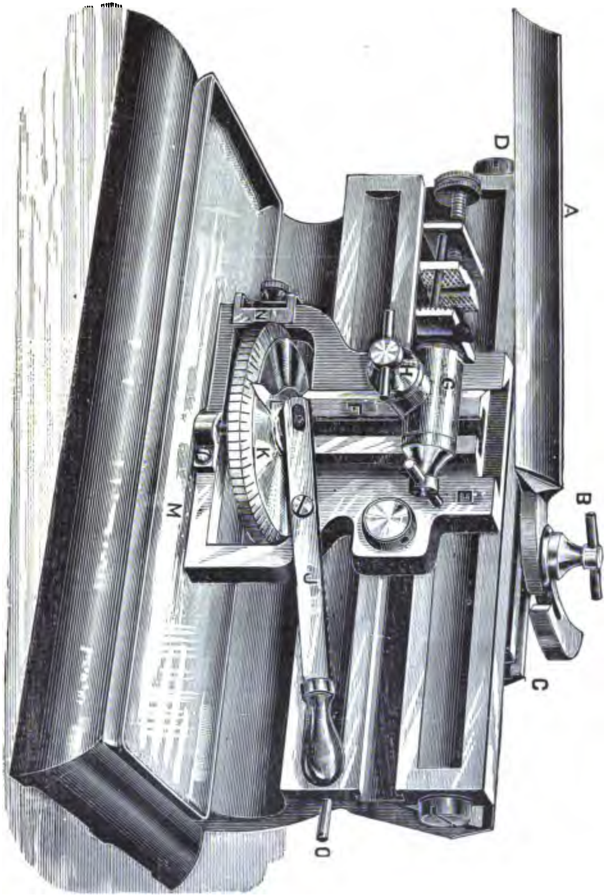

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